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(54) Title: Flt-3 LIGAND-ENCODING POLYNUCLEOTIDE AS A POLYNUCLEOTIDE-BASED VACCINE ENHANCER

(57) **Abstract:** The present invention provides a pharmaceutical composition, comprising a first non-infectious, non-integrating nucleic acid molecule construct comprising a polynucleotide encoding a Flt-3 ligand, and a second non-infectious, non-integrating nucleic acid molecule construct comprising a polynucleotide encoding an antigen or a cytokine. The present invention also provides a method broadly directed to enhancing immune response of a vertebrate in need of immunotherapy which entails administering *in vivo*, into a tissue of a vertebrate, a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides. The polynucleotides are incorporated into the cells of the vertebrate *in vivo*, and a prophylactically or therapeutically effective amount of a Flt-3 ligand and one or more antigens is produced *in vivo*.

Flt-3 Ligand-encoding Polynucleotide as a Polynucleotide-based Vaccine Enhancer

Field of the Invention

The present invention relates generally to immunogenic compositions and to methods useful for polynucleotide-based vaccination to help protect a vertebrate from a disease, to treat a diseased vertebrate, or both.

More particularly, the immunogenic compositions of the present invention comprise a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides. The present invention further provides methods of enhancing the immune response of a vertebrate by administering to the tissues of a vertebrate a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides.

Background of the Invention

Fms-like tyrosine kinase (Flt-3 ligand, Lyman SD *et al., Cell* 75(6):1157-67 (1993)) has been shown to produce systemic immunity to tumor cells when injected as a protein (Chen K *et al., Cancer. Res.* 57:3511-3516 (1997)). Administration of 500 µg/kg/day of Flt-3 ligand protein for ten days protected animals challenged with a murine breast cancer cell line. This protection was transient and lasted for less than four weeks. When a retrovirus expressing Flt-3 ligand was used to infect the murine breast cancer cells before implantation into the mice, the mice developed a long-lived immunity to the breast cancer challenge. Repeated systemic injection of Flt-3 ligand protein into mice results in the proliferation and mobilization of CD34+ hematopoietic stem cells as well as an increase in the numbers of natural killer cells (NK), mature dendritic cells (DC), B-cells, T-cells and other hematopoietic cells.

Another study showed that mice injected with 10 µg of Flt-3 ligand protein per day for 19 days showed complete tumor regression when challenged with B10.2 fibrosarcoma cells. This effect was shown to be due to the stimulation of a tumor-specific CTL response as well as non-specific anti-tumor

effects possibly due to increased Natural Killer (NK) cell response (Lynch DH, *Crit. Rev. Immunol.* 18:99-107 (1998)).

Another study showed that co-injection of mouse Flt-3 ligand can increase the B- and T-cell responses to poorly immunogenic antigens, influence the quality of the immune response (e.g., change the class of antibody produced) to a specific antigen, and enable the elicitation of an immune response to an antigen that would otherwise induce tolerance (Pulendran, *et al., J. Exp. Med. 188*:2075-2082). The effect was hypothesized to be due to the induction of dendritic cells.

U.S. Patent No. 5,676,954 reports on the injection of genetic material, complexed with cationic liposome-carriers, into mice. U.S. Patent Nos. 4,897,355; 4,946,787; 5,049,386; 5,459,127; 5,589,466; 5,693,622; 5,580,859; 5,703,055; and International Patent Application No. PCT/US94/06069 (publication no. WO 04/9469) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466; 5,693,622; 5,580,859; 5,703,055, and international patent application no. PCT/US94/06069 (publication no. WO 04/9469) provide methods for delivering DNA-cationic lipid complexes to mammals.

There remains a need in the art for convenient, safe, and efficacious immunogenic compounds to treat or prevent vertebrate diseases. The present invention provides simple and safe yet effective immunogenic compounds and methods to prevent and/or treat vertebrate diseases using such immunogenic compounds.

Summary of the Invention

The present invention is broadly directed to enhancing immune response of a vertebrate in need of immunotherapy by administering *in vivo*, into a tissue of a vertebrate, a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides. The polynucleotides are incorporated into the cells of the vertebrate *in vivo*, and a prophylactically or therapeutically

effective amount of a Flt-3 ligand and one or more antigens or cytokines is produced *in vivo*.

The present invention provides a pharmaceutical composition comprising about 1 ng to 10 mg of nucleic acid molecule comprising a first polynucleotide selected from the group consisting of (a) a polynucleotide that hybridizes under stringent conditions to a reference nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, and SEQ ID NO:22, wherein the polynucleotide encodes a polypeptide that has immunity-enhancing activity when administered to a vertebrate; (b) a polynucleotide that encodes a first polypeptide which, except for at least one but not more than 20 amino acid substitutions, deletions, or insertions, is identical to a second polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235 of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (c) a polynucleotide that encodes an amino acid sequence that is at least 90%, preferably at least 95%, and more preferably at least 97% identical to a reference amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (d) a polynucleotide encoding a polypeptide comprising at least 15, preferably at least 30, more preferably at least 50, and

more preferably at least150 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (e) a polynucleotide that encodes a polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235 of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (f) a polynucleotide encoding three amino acid regions comprising amino acid residues 34 to 41 of SEQ ID NO:19 arranged consecutively, amino acid residues 107 to 113 of SEQ ID NO:19 arranged consecutively, and amino acid residues 142 to 150 of SEQ ID NO:19 arranged consecutively, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (g) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:19, is identical to amino acids 27 to 160 of SEQ ID NO:19, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; (h) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:23, is identical to amino acids 27 to 185 of SEQ ID NO:23, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; and

(i) the complement of any of said polynucleotides; and about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding one or more antigens, wherein said first and second polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof in a subject in need of immunotherapy. The first and second polynucleotides can be present in a single nucleic acid molecule construct, or on separate nucleic acid molecule constructs.

In one embodiment, the pharmaceutical composition of the present invention further comprises about 1 ng to 10 mg of a nucleic acid molecule comprising a third non-infectious and non-integrating polynucleotide encoding a cytokine, chemokine, or other immunity-enhancing compound, wherein the third polynucleotide is operably associated with control sequences which direct its expression.

The present invention also provides a method of enhancing immune response of a vertebrate, comprising administering into a tissue of the vertebrate (a) a nucleic acid molecule comprising a first non-infectious, non-integrating polynucleotide encoding a Flt-3 ligand, or an active variant, or fragment thereof as described herein, and (b) a nucleic acid molecule comprising a second non-infectious, non-integrating polynucleotide encoding one or more antigens (e.g. an antigenic protein) and/or cytokines, such that the polynucleotides are expressed *in vivo* in an amount effective to enhance the immune response of the vertebrate to the encoded antigen or cytokine. The first and second polynucleotides can be present in a single nucleic acid molecule construct, or in separate nucleic acid molecule constructs. In some embodiments, the nucleic acid molecule construct(s) can be complexed with a cationic lipid formulation as described herein. When the polynucleotide is complexed with a catonic lipid formulation, the formulation preferably comprises a catonic lipid mixed with a co-lipid.

Brief Description of the Figures

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same become better understood by reference to the following detailed description when considered in connection with the accompanying figures.

Figure 1 shows the plasmid map of VR1623 which is the bicistronic chimeric idiotype (Id) plasmid DNA, referred to as pId, and having the nucleotide sequence SEQ ID NO:1. The cytomegalovirus immediate-early gene promoter, enhancer, and 5' untranslated sequences (5'UTR + intron A) drive the expression of the chimeric immunoglobulin light and heavy chain coding sequences. The transcriptional terminator region includes polyadenylation and termination signals derived from the bovine growth hormone gene. This plasmid expresses a functional chimeric Id immunoglobulin that stimulates a partially protective B and T cell response to the tumor.

Figure 2 shows the plasmid map of VR1605 which is the bicistronic plasmid containing the human immunoglobulin kappa light and gamma 1 heavy chain constant region DNA, referred to as pNegative, and having the nucleotide sequence SEQ ID NO:2. The plasmid contains the cytomegalovirus immediate-early gene promoter, enhancer, and 5' untranslated sequences (5'UTR + intron A) followed by the immunoglobulin light and heavy chain constant region coding sequences. The transcriptional terminator region includes polyadenylation and termination signals derived from the bovine growth hormone gene. This plasmid DNA (pDNA) contains the human constant regions only and not the mouse variable region sequences. This plasmid does not express a functional protein and therefore does not generate anti-Id antibodies or protect mice from 38C13 tumor challenge. It is used as a negative control for VR1623.

Figure 3 shows the plasmid map of VR 1642 (VAXID plasmid) which is the patient-specific bicistronic chimeric idiotype (Id) plasmid DNA used to treat B-cell lymphoma patients. This plasmid has the nucleotide sequence SEQ ID

NO:3. The cytomegalovirus immediate-early gene promoter, enhancer, and 5' untranslated sequences (5'UTR + intron A) drive the expression of the chimeric immunoglobulin light and heavy chain coding sequences. The human light and heavy chain variable regions are derived from each patient's tumor immunoglobulin sequence. VR1642 contains the light and heavy chain variable regions from the cell line RAMOS (Klein G *et al.,* Intervirology, 5:319-334 (1975); GenBank accession numbers Z74694 and Z74695) fused to the murine kappa light chain and gamma 1 heavy chain constant regions. The transcriptional terminator region includes polyadenylation and termination signals derived from the bovine growth hormone gene. This plasmid expresses a functional chimeric Id immunoglobulin to stimulate a B and T cell response to the patient's tumor.

Figure 4 shows VR1632 which is the bicistronic plasmid containing the murine immunoglobulin kappa light and gamma 1 heavy chain constant region DNA, having the nucleotide sequence SEQ ID NO:4. The plasmid contains the cytomegalovirus immediate-early gene promoter, enhancer, and 5' untranslated sequences (5'UTR + intron A) followed by the immunoglobulin light and heavy chain constant region coding sequences. The transcriptional terminator region includes polyadenylation and termination signals derived from the bovine growth hormone gene. This pDNA only contains the murine constant regions without any human variable region sequences. Tumor-specific variable regions from each patient are PCR amplified and cloned into this vector to produce each patient's vaccine construct.

Figure 5 shows the plasmid map of the expression plasmid VR1051, referred to as pBlank, and having the nucleotide sequence SEQ ID NO:5. The plasmid contains the cytomegalovirus immediate-early gene promoter, enhancer, and 5' untranslated sequences (5'UTR + intron A) followed by a multiple cloning site for inserting genes to be expressed. The transcriptional terminator region includes polyadenylation and termination signals derived from the rabbit beta globin gene. VR1051 is the expression plasmid without any insert and is used as a DNA mass control for VR6200.

Figure 6 shows the plasmid map of VR6200, which expresses a secreted form of the murine Flt-3 ligand. This plasmid is referred to herein as pFlt-3 lig, and has the nucleotide sequence SEQ ID NO:6. The Flt-3 ligand polypeptide encoded by this construct has been truncated to remove the transmembrane domain and cytoplasmic tail, leading to a secreted form of Flt-3 ligand. The insert is cloned as an *Eco*RI- *Nhe*I fragment in the VR1051 vector.

Figure 7 is a tumor cell survival curve demonstrating that the co-injection of a Flt-3 ligand-encoding plasmid with a tumor-specific antigen-encoding plasmid enhances protection from tumor challenge.

Figure 8 is a bar graph demonstrating that the co-injection of the Flt-3 ligand-encoding plasmid with an Id antigen-encoding plasmid results in greatly enhanced anti-Id antibody titer. This graph shows the total IgG anti-Id antibody response in ng/ml after plasmid vaccination and prior to tumor challenge.

Figure 9 is a tumor cell survival curve demonstrating that pFlt-3 ligand, while increasing the immune response to the co-injected plasmid (i.e., pID), does not have an effect by itself.

Detailed Description of Preferred Embodiments of the Invention

It will be apparent to one skilled in the art, in view of the following detailed description and the claims appended hereto, that various substitutions and modifications can be made to the present invention without departing from the scope of the invention as claimed.

The present invention is broadly directed to enhancing the immune response of a vertebrate to an antigen or a cytokine by administering *in vivo*, into a tissue of a vertebrate, a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides. The polynucleotides are incorporated into the cells of the vertebrate *in vivo*, and a prophylactically or therapeutically effective amount of a Flt-3 ligand and one or more antigens or cytokines is produced *in vivo*.

In one embodiment, the present invention provides a pharmaceutical composition comprising about 1 ng to 10 mg of a nucleic acid molecule comprising a first polynucleotide selected from the group consisting of: (a) a polynucleotide that hybridizes under stringent conditions to a reference nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, and SEQ ID NO:22, wherein the polynucleotide encodes a polypeptide that has immunity-enhancing activity when administered to a vertebrate; (b) a polynucleotide that encodes a first polypeptide which, except for at least one but not more than 20 amino acid substitutions, deletions, or insertions, is identical to a second polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235

of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (c) a polynucleotide that encodes an amino acid sequence that is at least 90%, preferably at least 95%, and more preferably at least 97% identical to a reference amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (d) a polynucleotide encoding a polypeptide comprising at least 15, preferably at least 30, more preferably at least 50, and more preferably at least150 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (e) a polynucleotide that encodes a polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235 of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (f) a polynucleotide encoding three amino acid regions comprising amino acid residues 34 to 41 of SEQ ID NO:19 arranged consecutively, amino acid residues 107 to 113 of SEQ ID NO:19 arranged consecutively, and amino acid residues 142 to 150 of SEQ ID NO:19 arranged

consecutively, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (g) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:19, is identical to amino acids 27 to 160 of SEQ ID NO:19, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; (h) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:23, is identical to amino acids 27 to 185 of SEQ ID NO:23, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; and (i) the complement of any of said polynucleotides; about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding one or more antigens or cytokines; and a pharmaceutically-acceptable carrier, wherein said first and second polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof in a subject in need of immunotherapy.

In another embodiment, the present invention provides an immunogenic composition obtained by mixing about 1 ng to 10 mg of a nucleic acid molecule comprising a polynucleotide selected from the group consisting of: (a) a polynucleotide that hybridizes under stringent conditions to a reference nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, and SEQ ID NO:22, wherein the polynucleotide encodes a polypeptide that has immunity-enhancing activity when administered to a vertebrate; (b) a polynucleotide that encodes a first polypeptide which, except for at least one but not more than 20 amino acid substitutions, deletions, or insertions, is identical to a second polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino

acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235 of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (c) a polynucleotide that encodes an amino acid sequence that is at least 90%, preferably at least 95%, and more preferably at least 97% identical to a reference amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (d) a polynucleotide encoding a polypeptide comprising at least 15, preferably at least 30, more preferably at least 50, and more preferably at least150 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (e) a polynucleotide that encodes a polypeptide selected from the group consisting of: amino acids 1 to 163, or 28 to 163 of SEQ ID NO:8, amino acids 1 to 189 or 28 to 189 of SEQ ID NO:8, amino acids 1 to 231 or 28 to 231 of SEQ ID NO:8, amino acids 1 to 220, or 28 to 220 of SEQ ID NO:11, amino acids 1 to 232, or 28 to 232 of SEQ ID NO:13, amino acids 1 to 172, or 28 to 172 of SEQ ID NO:17, amino acids 1 to 160, or 27 to 160 of SEQ ID NO:19, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:19, amino acids 1 to 235, or 27 to 235 of SEQ ID NO:19, amino acids 1 to 178, or 27 to 178 of SEQ ID NO:21, amino acids 1 to 185, or 27 to 185 of SEQ ID NO:23, and amino acids 1 to 235, or 27 to 235 of

SEQ ID NO:23, wherein said polypeptide has immunity-enhancing activity when administered to a vertebrate; (f) a polynucleotide encoding three amino acid regions comprising amino acid residues 34 to 41 of SEQ ID NO:19 arranged consecutively, amino acid residues 107 to 113 of SEQ ID NO:19 arranged consecutively, and amino acid residues 142 to 150 of SEQ ID NO:19 arranged consecutively, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate; (g) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:19, is identical to amino acids 27 to 160 of SEQ ID NO:19, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; (h) a polynucleotide encoding a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:23, is identical to amino acids 27 to 185 of SEQ ID NO:23, wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide; and (i) the complement of any of said polynucleotides; with one or more constructs comprising 1 ng to 30 mg of a nucleic acid molecule comprising one or more polynucleotides encoding one or more antigens or cytokines, wherein said polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof in a subject in need of immunotherapy.

The present invention also provides a method of enhancing the immune response of a vertebrate, comprising administering into a tissue of the vertebrate an immunogenic composition comprising (1) about 1 ng to 10 mg of a nucleic acid molecule comprising a first non-infectious, non-integrating polynucleotide encoding a Flt-3 ligand, or an active variant, or fragment thereof as described herein, and (2) about 1 ng to 30 mg of a nucleic acid molecule comprising a second non-infectious, non-integrating polynucleotide encoding one or more antigens (e.g. an antigenic protein) or cytokines, such that the polynucleotides are

expressed *in vivo* in an amount effective to enhance the immune response of the vertebrate to the encoded antigen or cytokines.

One example of a preferred embodiment of the present invention provides for the prophylactic or therapeutic treatment of B-cell lymphoma. This embodiment provides immunogenic compositions comprising (1) about 1 ng to 10 mg of a nucleic acid molecule comprising a first polynucleotide encoding a Flt-3 ligand; and (2) about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding a variable region of an immunoglobulin expressed by the B-cell lymphoma, including tumor-specific idiotype determinants, and a constant region. The immunogenic composition is administered into a tissue of a mammal, wherein specific anti-Id antibody responses are induced and such response is enhanced by the encoded Flt-3 ligand.

The preferred amount for both polynucleotides for this embodiment is about 10 μg to 10 mg, and the more preferred amount is about 0.1 mg to 5 mg, and the most preferred amount is about 0.5 mg to 2.0 mg.

The term “nucleic acid molecule” is intended to encompass a singular "nucleic acid molecule" as well as plural "nucleic acid molecules," and refers to an isolated molecule or construct comprising any one or more polynucleotides. The term “polynucleotide” refers to any one or more nucleic acid segments or constructs (e.g., DNA or RNA oligomers, mRNA or pDNA). The polynucleotide may be provided in linear, circular (e.g., plasmid), or branched form as well as double-stranded or single-stranded form. The polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)).

The first and second polynucleotides of the present invention can be present in a single nucleic acid molecule construct, e.g., on a single plasmid, or in separate nucleic acid molecule constructs, e.g., on separate plasmids. Furthermore, either the first or second polynucleotide may encode a single protein, e.g., a single antigen, cytokine, or a Flt-3 ligand, or may encode more than one protein, e.g., a nucleic acid molecule may comprise a second

polynucleotide encoding two or more antigens and/or cytokines, either as separate proteins or as a fusion protein, or a nucleic acid molecule may comprise a first polynucleotide encoding a Flt-3 ligand and a second polynucleotide encoding one or more antigens and/or cytokines, either as separate proteins or as one or more fusion proteins. The first and second polynucleotides may also encode a single fusion protein which combines one or more antigens and/or cytokines and a Flt-3 ligand, active variant, or fragment thereof as described herein.

Immunogenic compositions of the present invention include a nucleic acid molecule comprising at least one polynucleotide encoding an antigen or a cytokine. In certain embodiments of the present invention, the immunogenic compositions can include multiple antigen- or cytokine-encoding polynucleotides. The term "antigen" is intended to encompass a singular "antigen" as well as plural "antigens." As used herein, an "antigen" is any compound comprising at least one epitope. An "epitope," is the minimum portion of an antigen required to interact with an immune molecule generated by a vertebrate, e.g., with an antibody or a T-cell receptor. Antigens and their epitopes can comprise, for example, carbohydrates, lipids, nucleic acids, amino acids, and a wide range of synthetic organic chemicals. An antigen of the present invention is encoded by a polynucleotide, and accordingly, comprises either nucleic acids or amino acids. However, it is within the scope of the present invention for an antigen comprised of amino acids to incorporate, during its synthesis *in vivo*, modifications including, but not limited to, the addition of carbohydrate antigens and lipid antigens. Preferably, an antigen of the present invention is a protein antigen, i.e., it comprises epitopes composed of amino acids. The minimum size of a protein epitope is generally understood by those skilled in the art to be about 6-12 amino acids, either consecutive on the amino acid chain, i.e., a continuous epitope, or formed through the interactions of non-consecutive amino acid residues due to the secondary or tertiary structure of a protein, i.e., a discontinuous epitope. *See*, e.g., Harlow and Lane, *Antibodies A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1988), and

Janeway, *et al.*, *Immunobiology: Immune System in Health and Disease*, Current Biology Publication, New York, New York (1999), both of which are incorporated herein by reference.

In certain embodiments of the present invention, the immunogenic compositions can include one or more cytokine-encoding polynucleotides. The term "cytokine" is intended to encompass a singular "cytokine" as well as plural "cytokines." Examples of cytokines include, but are not limited to granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin 3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interleukin 15 (IL-15), interleukin 18 (IL-18), interferon alpha (IFNα), interferon beta (IFNβ), interferon gamma (IFNγ), interferon omega (IFNω) and interferon tau (IFNτ), interferon gamma inducing factor (IGIF), transforming growth factor beta (TGF-β) RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), *Leishmania* elongation initiating factor (LEIF), stromal cell derived factor 1 (SDF-1), and monocyte chemotactic protein-3.

The term "vertebrate" is intended to encompass a singular "vertebrate" as well as plural "vertebrates," and comprises mammals and birds, as well as fish, reptiles, and amphibians.

The term "mammal" is intended to encompass a singular "mammal" and plural "mammals," and includes, but is not limited to humans; primates such as apes, monkeys, orangutans, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras, food animals such as cows, pigs, and sheep; ungalates such as deer and giraffes; and bears. Preferably, the mammal is a human subject.

As used herein, an "immune response" refers to the ability of a vertebrate to elicit an immune reaction to a composition delivered to that vertebrate.

Examples of immune responses include an antibody response or a cellular, e.g., T-cell, response. An immunogenic composition of the present invention may be used to treat a vertebrate prophylactically, e.g., as a prophylactic vaccine, to establish or enhance immunity in a healthy vertebrate prior to exposure or contraction of a disease or disorder, thus preventing the disease or reducing the severity of disease symptoms or to reducing the severity of a metabolic disorder. An immunogenic composition of the present invention may also be used to treat a vertebrate already suffering from a disease or a metabolic disorder to further stimulate the immune system of the vertebrate, thus reducing or eliminating the symptoms associated with that disease or disorder. As defined herein, "treatment of a vertebrate" refers to the use of an immunogenic composition of the present invention to prevent, cure, retard, or reduce the severity of disease symptoms in a vertebrate; to reduce tumor size, reduce the rate of metastasis, and/or slow tumor growth, and/or result in no worsening in disease over a specified period of time. It is not required that an immunogenic composition of the present invention provide total immunity or totally cure or eliminate all disease symptoms. As used herein, a “subject in need of immunotherapy” refers to a vertebrate which it is desirable to treat, i.e., to prevent, cure, retard, or reduce the severity of disease symptoms; to reduce tumor size, reduce the rate of metastasis, and/or slow tumor growth, and/or result in no worsening in disease over a specified period of time.

In some circumstances, an antigen of the present invention is capable of eliciting, without more, an immune response in a vertebrate when administered to that vertebrate. Such antigens are referred to herein as immunogenic antigens. However, antigens that do not normally elicit an immune response upon administration to a vertebrate, i.e., non-immunogenic antigens, are also within the scope of the present invention. Indeed, one advantage of the present invention is the ability to elicit or greatly enhance an immune response in a vertebrate to a non-immunogenic antigen, for example, an antigen to which the vertebrate is tolerant, when a polynucleotide encoding that antigen is co-administered with a

polynucleotide encoding a Flt-3 ligand or an active variant, or fragment thereof as described herein.

Antigens that normally do elicit an immune response in a vertebrate, i.e., "immunogens," are also within the scope of the present invention. The term "immunogen" is meant to encompass any compound capable of eliciting an immune response in a vertebrate.

An antigen of the present invention includes any compound that is capable of being encoded by a polynucleotide, for example, a polypeptide, DNA, or RNA molecule. Preferably, an antigen of the present invention comprises a polypeptide. Such an antigen may comprise one or more complete proteins, or may be a fragment of a protein. At a minimum, a polypeptide antigen of the present invention must include at least one epitope capable of interaction with the immune system of a vertebrate. In one preferred embodiment, a polypeptide antigen of the present invention is derived from a heterologous organism, such as a pathogen. Other preferred polypeptide antigens of the present invention include allergens, tumor-associated antigens, and self-antigens linked to cells associated with autoimmune diseases. Examples of suitable antigens are disclosed herein.

In a preferred embodiment, the present invention can be applied to the treatment of B-cell lymphoma. In one example of this embodiment, the antigen-encoding polynucleotide comprises a polynucleotide encoding a variable region of a B-cell lymphoma immunoglobulin having tumor-specific idiotype determinants. An "idiotype determinant" is the region of an immunoglobulin molecule that directly and specifically binds to an epitope of a given antigen. The polynucleotide can further encode a constant region. The antigen-encoding polynucleotide can be a polycistronic plasmid DNA (pDNA). As defined herein, a “cistron” is a translational coding region, and “polycistronic” refers to a polynucleotide comprising two or more “cistrons.” In one embodiment, an antigen-encoding polycistronic polynucleotide comprises a first cistron, encoding a variable light chain region of a B-cell lymphoma immunoglobulin with a tumor-

specific idiotype determinant fused in-frame to a light chain constant region and a second cistron encoding a variable heavy chain region of a B-cell lyphoma immunoglobulin with a tumor-specific idiotype determinant, fused in-frame to a heavy chain constant region. A preferred heavy or light chain constant region is a xenogeneic constant region (i.e., a constant region derived from a heterologous species relative relative to the variable region). For example, if a variable region is derived from mouse, it is fused in-frame to a constant region derived from human. The cistrons can be organized in a transcription unit under the control of a single promoter, and said polynucleotide can further comprise an internal ribosome entry site positioned between the cistrons. Suitable internal ribosome entry sites are disclosed, for example, in Duke, *et al.*, *J. Virol. 66*:1602-1609 (March, 1992), and Gan and Rhoads, *J. Biol. Chem. 271*:623-626 (January, 1996). In this embodiment, the antigen- or cytokine-encoding polynucleotides or the immunogenic compositions containing the polynucleotides are preferably custom made to express the idiotype determinants of each patient's own tumor immunoglobulin (Ig).

In one embodiment, the present invention comprises a nucleic acid molecule comprising a first polynucleotide that hybridizes under stringent conditions to a reference nucleic acid molecule that encodes a Flt-3 ligand, or a fragment or variant thereof as described herein, or a complement of any such reference nucleic acid molecule, wherein the first polynucleotide encodes a polypeptide that has immunity-enhancing activity when administered to a vertebrate. "Stringent hybridization conditions" are those experimental parameters that allow an individual skilled in the art to identify similarities between heterologous nucleic acid molecules. *See*, for example, Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, Cold Spring Harbor, NY (1989), and Meinkoth, *et al., Anal. Biochem.* 138:267-284 (1984), both of which are incorporated herein by reference. The determination of stringent hybridization conditions involves the manipulation of a set of variables, including ionic strength (M, in moles/liter), the hybridization temperature (°C),

the concentration of helix destabilizing agents (such as formamide), the average length of the shortest chain in the duplex (n), and the percent G + C composition of the fragments being hybridized. For nucleic acid molecules longer than about 50 nucleotides, these variables are inserted into a standard formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule, which is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

$$T_m=81.5\ ^{\circ}C + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%formamide).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability, at a standard ionic strength, is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

It is also well known by those skilled in the art how base-pair mismatch will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1 °C for each 1% of mismatched base-pairs for hybrids greater than about 150 base pairs (bp), and T_d decreases about 5 °C for each mispaired base-pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base-pairs can be determined empirically. This allows one skilled in the art to set the hybridization conditions (by altering, for example, the salt concentration, the formamide concentration or the temperature) such that only hybrids with greater than a specified % base-pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow no more than about 3-5% base-pair mismatch (i.e., about 95-97% identity between the hybrid strands). "Stringent conditions" for a hybridization probe larger than 100 nucleotides, for example, may comprise hybridization by overnight incubation at 42°C in a solution comprising: 50% formamide, 5x SSC (750 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran

sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by repeatedly washing the filters (at least three times) in 0.1x SSC and 0.1% sodium dodecyl sulfate (w/v) for 20 minutes at about 65°C. Using the formula described above with a probe of about 1000 nucleotides, having an about 40% G + C content, the Tm of fully complementary hybrids will be about 67 °C. Thus, the stringent wash at 65 °C will allow detection of hybrids having 2% or less base-pair mismatch.

It will be recognized in the art that some amino acid sequences of Flt-3 ligand and antigen- or cytokine-encoding polypeptides described herein can be varied without significant effect on the functional activity of the polypeptides. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the polypeptide which determine activity. Such variations include deletions, insertions, inversions, repeats, and type substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., *et al.*, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," (*Science 247*:1306-1310 (1990)), which is incorporated herein by reference in its entirety. Polypeptides suitable for use in the present invention can be assayed by methods known to those skilled in the art, examples of which are described in the Examples, *infra*. Amino acids that are critical for, e.g., cytokine activity can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, *et al., J. Mol. Biol. 224*:899-904 (1992) and de Vos, *et al. Science 255*:306-312 (1992)).

To encompass variants of a Flt-3 ligand containing suitable deletions, insertions, inversions, repeats, and type substitutions, one embodiment of the present invention provides a nucleic acid molecule comprising a first polynucleotide that encodes an amino acid sequence that is at least 90%, preferably at least 95%, and more preferably at least 97% identical to a reference amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ

ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:23, wherein said polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate. As a practical matter, whether any particular amino acid sequence is at least 90%, 95%, or 97%, identical to, for instance, reference amino acid sequences having the above SEQ ID NOs can be determined conventionally using known computer programs such as the Bestfit program with default parameters (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, Wis. 5371 1). Bestfit uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences.

The present invention further relates to using variants of Flt-3 ligand and antigen- or cytokine-encoding polynucleotides, which encode portions, analogs or derivatives of a Flt-3 ligand, antigen, or cytokine, wherein the portions, analogs, or derivatives of a Flt-3 ligand retain immunity-enhancing activity, and wherein the portions, analogs, or derivatives of an antigen retain at least one epitope. Variants may occur naturally, such as a natural allelic variant or a splice variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. *Genes II*, Lewin, B., ed., John Wiley & Sons, New York (1985). A "splice-variant" is a messenger RNA or gene product produced by the joining of alternative exons upon the processing of transcripts of a common gene. Examples of naturally-occurring allelic variants and splice variants of polynucleotides encoding a Flt-3 ligand are disclosed herein. Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Examples of allelic variants and splice variants of polynucleotides encoding full-length Flt-3 ligands include, for example, polynucleotides having nucleic acid sequences SEQ ID NO:7, encoding amino acid sequence SEQ ID NO:8; SEQ ID NO:10, encoding amino acid sequence SEQ ID NO:11; SEQ ID NO:12, encoding amino acid sequence SEQ ID NO:13; SEQ ID NO:14, encoding

amino acid sequence SEQ ID NO:15; SEQ ID NO:16, encoding amino acid sequence SEQ ID NO:17; SEQ ID NO:18, encoding amino acid sequence SEQ ID NO:19; SEQ ID NO:20, encoding amino acid sequence SEQ ID NO:21; and SEQ ID NO:22, encoding amino acid sequence SEQ ID NO:23.

In one naturally-occurring form, a mature Flt-3 ligand protein is bound to the surface of cells and comprises an extracellular domain, a transmembrane domain and a cytoplasmic domain. The full-length protein further comprises an N-terminal signal peptide. *See*, e.g., Graddis, *et al.*, *J. Biol. Chem., 273*:17626-17633 (1998), and U.S. Patent No. 5,554,512.

It is believed that the N-terminal signal peptide of a full-length Flt-3 ligand polypeptide comprises the first 26 or 27 amino acids of that protein, e.g., amino acids 1-27 of SEQ ID NO:8 and SEQ ID NO:9, encoded by nucleotides 32-112 of SEQ ID NO:7; amino acids 1-27 of SEQ ID NO:11, encoded by nucleotides 7-87 of SEQ ID NO:10; amino acids 1-27 of SEQ ID NO:13, encoded by nucleotides 256-336 of SEQ ID NO:12; amino acids 1-27 of SEQ ID NO:15, encoded by nucleotides 1-81 of SEQ ID NO:14; amino acids 1-27 of SEQ ID NO:17, encoded by nucleotides 1-81 of SEQ ID NO:16; amino acids 1-26 of SEQ ID NO:19, encoded by nucleotides 84-161 of SEQ ID NO:18; amino acids 1-26 of SEQ ID NO:21, encoded by nucleotides 1-78 of SEQ ID NO:20; and amino acids 1-26 of SEQ ID NO:23 and SEQ ID NO:24, encoded by nucleotides 93-170 of SEQ ID NO:22.

Examples of mature Flt-3 ligand polypeptides, i.e., where the N-terminal signal peptide has been removed, include a polypeptide having amino acids 28-231 of SEQ ID NO:8, encoded by nucleotides 113-724 of SEQ ID NO:7; a polypeptide having amino acids 28-189 of SEQ ID NO:9, encoded by nucleotides 113-598 of SEQ ID NO:7; a polypeptide having amino acids 28-220 of SEQ ID NO:11, encoded by nucleotides 88-666 of SEQ ID NO:10; amino acids 28-232 of SEQ ID NO:13, encoded by nucleotides 337-951 of SEQ ID NO:12; amino acids 28-220 of SEQ ID NO:15, encoded by nucleotides 82-660 of SEQ ID NO:14; amino acids 28-172 of SEQ ID NO:17, encoded by nucleotides 82-516

of SEQ ID NO:16; amino acids 27-235 of SEQ ID NO:19, encoded by nucleotides 162-788 of SEQ ID NO:18; amino acids 27-178 of SEQ ID NO:21, encoded by nucleotides 79-534 of SEQ ID NO:20; amino acids 27-235 of SEQ ID NO:23 encoded by nucleotides 171-797 of SEQ ID NO:22; and amino acids 27-185 of SEQ ID NO:24, encoded by nucleotides 171-647 of SEQ ID NO:22.

It is believed that the extracellular domain of a membrane bound Flt-3 ligand polypeptide comprises about 151-156 amino acids, e.g., from about amino acid 28 to about amino acid 188 of SEQ ID NO:8, encoded by nucleotides 113-595 of SEQ ID NO:7; from about amino acid 28 to about amino acid 188 of SEQ ID NO:13, encoded by nucleotides 337-819 of SEQ ID NO:12; from about amino acid 27 to about amino acid 182 of SEQ ID NO:19, encoded by nucleotides 162-629 of SEQ ID NO:18; and from about amino acid 27 to about amino acid 182 of SEQ ID NO:23, encoded by nucleotides 170-638 of SEQ ID NO:22.

It is believed that the transmembrane domain of a membrane-bound Flt-3 ligand polypeptide comprises about 23-24 amino acids, e.g., from about amino acid 189 to about amino acid 211 of SEQ ID NO:8, encoded by nucleotides 596-664 of SEQ ID NO:7; from about amino acid 189 to about amino acid 212 of SEQ ID NO:13, encoded by nucleotides 820-891 of SEQ ID NO:12; from about amino acid 183 to about amino acid 205 of SEQ ID NO:19, encoded by nucleotides 630-708 of SEQ ID NO:18; and from about amino acid 183 to about amino acid 205 of SEQ ID NO:23, encoded by nucleotides 639-697 of SEQ ID NO:22.

It is believed that the cytoplasmic domain of a membrane-bound Flt-3 ligand polypeptide comprises about 20-30 amino acids, e.g., from about amino acid 212 to about amino acid 231 of SEQ ID NO:8, encoded by nucleotides 665-724 of SEQ ID NO:7; from about amino acid 213 to about amino acid 232 of SEQ ID NO:13, encoded by nucleotides 892-951 of SEQ ID NO:12; from about amino acid 206 to about amino acid 235 of SEQ ID NO:19, encoded by nucleotides 699-788 of SEQ ID NO:18; and from about amino acid 206 to about

amino acid 235 of SEQ ID NO:23, encoded by nucleotides 709-797 of SEQ ID NO:22.

Furthermore, a soluble mature Flt-3 ligand, lacking the secretory signal peptide, transmembrane and cytoplasmic domains, is biologically active. *See* McClanahan, *et al., Blood* 88: 3371-3382 (1996), which is incorporated herein by reference. Accordingly, a preferred variant of a polynucleotide encoding a Flt-3 ligand is a polynucleotide encoding a secreted form of a Flt-3 ligand. Naturally-occurring secreted forms of a Flt-3 ligand may be encoded by a polynucleotide comprising a splice variant lacking the coding regions for the transmembrane and cytoplasmic domains, e.g., a Flt-3 ligand encoded by the polynucleotide having SEQ ID NO:10, encoding a full length polypeptide having the amino acid sequence SEQ ID NO:11; or a Flt-3 ligand encoded by the polynucleotide having SEQ ID NO:16, encoding a full length polypeptide having the amino acid sequence SEQ ID NO:17; or may be formed by post-translational processing of a membrane bound form of Flt-3 ligand. *See*, e.g., McClanahan, *et al.,ibid.* Alternatively, a polynucleotide encoding a secreted form of a Flt-3 ligand may be constructed through standard cloning techniques. *See*, e.g., Examples 1 and 2, *infra.* Examples of constructed polynucleotides encoding full-length secreted forms of Flt-3 ligand include a polynucleotide having nucleotides 32-598 of SEQ ID NO:7, encoding amino acid sequence SEQ ID NO:9, and a polynucleotide having nucleotides 93-647 of SEQ ID NO:22, encoding amino acid sequence SEQ ID NO:24.

Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the Flt-3 ligand, antigen, or portions thereof. Also especially preferred in this regard are conservative substitutions. For example, aromatic amino acids that can be conservatively substituted for one another include phenylalanine, tryptophan, and tyrosine. Hydrophobic amino acids that can be conservatively substituted for one another include leucine,

isoleucine, and valine. Polar amino acids that can be conservatively substituted for one another include glutamine and asparagine. Basic amino acids that can be conservatively substituted for one another include arginine, lysine, and histidine. Acidic amino acids that can be conservatively substituted for one another include aspartic acid and glutamic acid. Small amino acids that can be conservatively substituted for one another include alanine, serine, threonine, methionine, and glycine.

Substitutions, deletions, or insertions can be made outside of the region encoding the shortest active fragment of the Flt-3 ligand or outside of the epitopes contained in the antigen, without affecting the activity of the immunogenic compound of the present invention. Further, mutated proteins (or muteins) often retain a biological activity that is similar to that of the naturally occurring protein. For example, Gayle and coworkers (*J. Biol. Chem. 268:* 22105-22111 (1993)) conducted an extensive mutational analysis of the human cytokine IL-1α. They used random mutagenesis to generate over 3,500 individual IL-1α mutants with an average of 2.5 amino acid changes per mutein over the entire length of the molecule. Multiple mutations were examined at every possible amino acid and, on average, each mutein's amino acid sequence was 98.4% identical to that of naturally occurring IL-1α. The investigators observed that most of the molecule could be mutated with little effect on either binding or biological activity, and that 75% of the molecule may not contribute significantly to the biological activity of the molecule.

More specifically, Graddis, *et al.*, *ibid.*, reported a random mutational analysis of the human Flt-3 ligand, screening over 60,000 potential mutants for receptor binding and biological activity. They observed that mutations in three "hot spots" on the Flt-3 ligand polypeptide, amino acids 34 to 41, 107 to 113, and 142 to 150 of the full-length polypeptide i.e., SEQ ID NO:19 or SEQ ID NO:23, either enhanced or reduced biological activity, and that in the predicted three-dimensional structure of the molecule, these regions cluster together as a small surface patch, which they hypothesize to be the receptor binding site. Graddis

and coworkers showed that certain non-conservative substitutions in the full-length polypeptide enhanced biological activity. These included substitutions, e.g., in polypeptides having amino acid sequences SEQ ID NO:19 or SEQ ID NO:23, at amino acid positions: His-34, e.g., substituted with Tyr; Lys-110, e.g., substituted with either Glu or Thr; Trp-144, e.g., substituted with Arg; and Gln-148, e.g., substituted with Arg. Graddis and coworkers found further substitutions that reduced, but did not eliminate activity. Two other mutations that reduced biological activity of the protein, at amino acid positions Lys-53 and Ala-90, mapped to the proposed dimerization interface, shifting the equilibrium of the population of Flt-3 ligand proteins from dimers to monomers. Mutations in other regions of the protein appeared to have no appreciable effect on activity of the molecule. Thus, even non-conservative substitutions could be made in these other regions without appreciably affecting activity of the Flt-3 ligand polypeptide.

Accordingly, a polynucleotide encoding a Flt-3 ligand or antigen of the present invention can encode a polypeptide having one to about twenty amino acid substitutions, deletions or insertions, either from natural mutations or human manipulation, relative to the full length, mature or secreted form of Flt-3 ligand or antigen. Preferably, no more than one to fifteen substitutions, deletions or insertions are present, relative to the full length, mature or secreted form of Flt-3 ligand or antigen, in addition to normal truncation of the transmembrane domain, cytoplasmic tail, and the secretory signal peptide sequence. More preferably, no more than one to ten substitutions, deletions or insertions are present. Still more preferably, no more than one to five substitutions, deletions or insertions are present. Even more preferably only one substitution, deletion or insertion is present.

Especially preferred is a polynucleotide encoding a human Flt-3 ligand of the present invention having one to about twenty amino acid substitutions, deletions or insertions in regions outside of the three "hot spots" on the Flt-3 ligand polypeptide, i.e., the substitutions, deletions, or insertions do not occur in regions identical to amino acids 34 to 41, 107 to 113, and 142 to 150 of a

polypeptide having amino acid sequence SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:24. Preferably, no more than one to fifteen substitutions, deletions or insertions are present outside of these regions, relative to the full length, mature or secreted form of a Flt-3 ligand, in addition to normal truncation of the transmembrane domain, cytoplasmic tail, and the leader sequence. More preferably, no more than one to ten substitutions, deletions, or insertions are present. Still more preferably, no more than one to five substitutions, deletions, or insertions are present outside of these regions. Even more preferably, only one substitution, deletion, or insertion is present outside of these regions.

Also especially preferred is a polynucleotide encoding a human Flt-3 ligand of the present invention having one or more activity-increasing substitutions at amino acid positions 34, 110, or 144, or 147 of a polypeptide having amino acid sequence SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, or SEQ ID NO:24.

Polynucleotide and amino acid sequences encoding a Flt-3 ligand include genomic sequences of human and murine Flt-3 ligand genes, as well as cDNA and protein sequences encoding full-length natural allelic variants and splice variants of human and murine Flt-3 ligands, mature human and murine Flt-3 ligands, and secreted forms of human and murine Flt-3 ligands, set forth, e.g., in U.S. Patent No. 5,554,512 , Lyman, *et al.*, *Oncogene* 11:1165-1172 (1995), Lyman, *et al.*, *Oncogene* 10:149-157 (1995), Hannum, *et al.*, *Nature* 368:643-648 (1994), Graddis, *et al., ibid.*, and McClanahan, *et al., ibid.*

Secretion of a peptide from a cell can be facilitated by a leader sequence, also referred to as a secretory signal peptide. In a preferred embodiment, either the native leader sequence is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the peptide that is operably associated with it. Alternatively, a heterologous mammalian leader sequence, or a functional derivative thereof, may be used. For example, the wild-type leader sequence

may be substituted with the leader sequence of human tissue plasminogen activator or mouse β-glucuronidase.

In one embodiment, the pharmaceutical composition of the present invention can further include one or more known adjuvants. The term "adjuvant" refers to any material having the ability to (1) alter or increase the immune response to a particular antigen or (2) increase or aid an effect of a pharmacological agent. Accordingly, a Flt-3 ligand encoded by a polynucleotide of the present invention is considered an "adjuvant." This embodiment refers to a pharmaceutical composition comprising one or more additional adjuvants. Suitable adjuvants include, but are not limited to, cytokines and growth factors; bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viruses and virally-derived materials, poisons, venoms, and catatonic lipids. The ability of an adjuvant to increase the immune response to an antigen is typically manifested by a significant increase in immune-mediated protection. For example, an increase in humoral immunity is typically manifested by a significant increase in the titer of antibodies raised to the antigen, and an increase in T-cell activity is typically manifested in increased cell proliferation, or cellular cytotoxicity. An adjuvant may also alter an immune response, for example, by changing a primarily humoral or Th_2 response into a primarily cellular, or Th_1 response.

The preferred adjuvants are cytokines. It is known in the art that the biological activity of Flt-3 ligand works synergistically with other cytokines and growth factors, to greatly increase the immunomodulatory effect. See, e.g., Shurin, *et al.*, *Cytokine Growth Factor Rev.* 9:37-48 (1998). A pharmaceutical composition of the present invention can comprise one or more cytokines, chemokines, or compounds that induce the production of cytokines and chemokines, or a polynucleotide encoding one or more cytokines, chemokines, or compounds that induce the production of cytokines and chemokines. Examples include, but are not limited to granulocyte macrophage colony stimulating factor

(GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interleukin 15 (IL-15), interleukin 18 (IL-18), interferon alpha (IFNα), interferon beta (IFNβ), interferon gamma (IFNγ), interferon omega (IFNω), interferon tau (IFNτ), interferon gamma inducing factor I (IGIF), transforming growth factor beta (TGF-β), RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and *Leishmania* elongation initiating factor (LEIF).

The immunogenic composition of the present invention can also include one or more transfection facilitating materials that facilitate delivery of polynucleotides to the interior of a cell, and/or to a desired location within a cell. Examples of the transfection facilitating materials include, but are not limited to inorganic materials such as calcium phosphate, alum (aluminum sulfate), and gold particles (e.g., "powder" type delivery vehicles); peptides that are, for example, cationic, intercell targeting (for selective delivery to certain cell types), intracell targeting (for nucleor localization or endosomal escape), and ampipathic (helix forming or pore forming); proteins that are, for example, basic (e.g., positively charged) such as histones, targeting (e.g., asialoprotein), viral (e.g., Sendai virus coat protein), and pore-forming; lipids that are, for example, cationic (e.g., DMRIE, DOSPA, DC-Chol), basic (e.g., steryl amine), neutral (e.g., cholesterol), anionic (e.g., phosphatidyl serine), and zwitterionic (e.g., DOPE, DOPC); and polymers such as dendrimers, star-polymers, "homogenous" poly-amino acids (e.g., poly-lysine, poly-arginine), "heterogenous" poly-amino acids (e.g., mixtures of lysine & glycine), co-polymers, polyvinylpyrrolidinone (PVP), and polyethylene glycol (PEG). A transfection facilitating material can be used alone or in combination with one or more other transfection facilitating materials. Two or more transfection facilitating materials can be combined by

chemical bonding (e.g., covalent and ionic such as in lipidated polylysine, PEGylated polylysine) (Toncheva, *et al.*, *Biochim. Biophys. Acta 1380(3)*:354-368 (1988)), mechical mixing (e.g., free moving materials in liquid or solid phase such as "polylysine + cationic lipids") (Gao and Huang, *Biochemistry 35*:1027-1036 (1996); Trubetskoy, *et al., Biochem. Biophys. Acta 1131*:311-313 (1992)), and aggregation (e.g., co-precipitation, gel forming such as in cationic lipids + poly-lactide co-galactide, and polylysine + gelatin).

The preferred transfection facilitating agents are cationic lipids, which can bind effectively to negatively charged molecules of DNA. Examples of cationic lipids are 5-carboxyspermylglycine dioctadecylamide (DOGS) and dipalmitoyl-phophatidylethanolamine-5carboxyspermylamide (DPPES). Cationic cholesterol derivatives are also useful, including {3β-[N-N',N'-dimethylamino)ethane]-carbomoyl}-cholesterol (DC-Chol). Dimethyldioctdecyl-ammonium bromide (DDAB), N-(3-aminopropyl)-N,N-(*bis*-(2-tetradecyloxyethyl))-N-methyl-ammonium bromide (PA-DEMO), N-(3-aminopropyl)-N,N-(*bis*-(2-dodecyloxyethyl))-N-methyl-ammonium bromide (PA-DELO), N,N,N-*tris*-(2-dodecyloxy)ethyl-N-(3-amino)propyl-ammonium bromide (PA-TELO), and N^1-(3-aminopropyl)((2-dodecyloxy)ethyl)-N^2-(2-dodecyloxy)ethyl-1-piperazinaminium bromide (GA-LOE-BP) can also be employed in the present invention.

Non-diether cationic lipids, such as DL-1,2-dioleoyl-3-dimethylaminopropyl-β-hydroxyethylammonium (DORI diester), 1-O-oleyl-2-oleoyl-3-dimethylaminopropyl-β-hydroxyethylammonium (DORI ester/ether), and their salts promote *in vivo* gene delivery. Preferred cationic lipids comprise groups attached via a heteroatom attached to the quaternary ammonium moiety in the head group. A glycyl spacer can connect the linker to the hydroxyl group.

Preferred cationic lipids for use in certain embodiments of the present invention include DMRIE ((±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide), GAP-DMORIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium

bromide), and GAP-DLRIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(*bis*-dodecyloxy)-1-propanaminium bromide).

A preferred cationic lipid of the present invention is a "cytofectin." As used herein, a "cytofectin" refers to a subset of cationic lipids which incorporate certain structural features including, but not limited to, a quaternary ammonium group and/or a hydrophobic region (usually with two or more alkyl chains), but which do not require amine protonation to develop a positive charge. Examples of cytofectins may be found, for example, in U.S. Patent No. 5,861,397.

Also preferred are (±)-N,N-dimethyl-N-[2-(sperminecarboxamido)ethyl]-2,3-bis(dioleyloxy)-1-propaniminium pentahydrochloride (DOSPA), (±)-N-(2-aminoethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propaniminium bromide (β-aminoethyl-DMRIE or βAE-DMRIE) (Wheeler, *et al., Biochim. Biophys. Acta 1280*:1-11 (1996)), and (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-l-propaniminium bromide (GAP-DLRIE) (Wheeler, *et al., Proc. Natl. Acad. Sci. USA 93*:11454-11459 (1996)), which have been developed from DMRIE.

Other examples of DMRIE-derived cationic lipids that are useful for the present invention are (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(*bis*-decyloxy)-1-propanaminium bromide (GAP-DDRIE), (±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(*bis*-tetradecyloxy)-1-propanaminium bromide (GAP-DMRIE), (±)-N-((N"-methyl)-N'-ureyl)propyl-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide (GMU-DMRIE), (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (DLRIE), and (±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-*bis*-([Z]-9-octadecenyloxy)propyl-1- propaniminium bromide (HP-DORIE).

Preferred cytofectins for use in certain embodiments of the present invention include DMRIE ((±)-N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide), GAP-DMORIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium

bromide), and GAP-DLRIE ((±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-(*bis*-dodecyloxy)-1-propanaminium bromide).

In the embodiments where the immunogenic composition comprises a cationic lipid, the cationic lipid is preferably mixed with one or more co-lipids. For purposes of definition, the term "co-lipid" refers to any hydrophobic material which may be combined with the cationic lipid component and includes amphipathic lipids, such as phospholipids, and neutral lipids, such as cholesterol. Cationic lipids and co-lipids may be mixed or combined in a number of ways to produce a variety of non-covalently bonded macroscopic structures, including, for example, liposomes, multilamellar vesicles, unilamellar vesicles, micelles, and simple films. A preferred class of co-lipids are the zwitterionic phospholipids, which include the phosphatidylethanolamines and the phosphatidylcholines. Most preferably, the co-lipids are phosphatidylethanolamines, such as, for example, DOPE, DMPE and DPyPE. DOPE and DPyPE are particularly preferred; the most preferred co-lipid is DPyPE, which comprises two phytanoyl substituents incorporated into the diacylphosphatidylethanolamine skeleton.

When the immunogenic composition comprises a cationic lipid:co-lipid, the preferred cationic lipid:co-lipid molar ratio of the present invention is from about 9:1 to about 1:9. More preferably, the cationic lipid:co-lipid molar ratio is from about 4:1 to about 1:4 and, still more preferably, is from about 2:1 to about 1:2. The most preferred molar ratio is about 1:1.

In order to maximize homogenity, the cationic lipid and co-lipid components of the immunogenic compositions in certain embodiments of the present invention are preferably dissolved in a solvent such as chloroform, followed by evaporation of the cationic lipid/co-lipid solution under vacuum to dryness as a film on the inner surface of a glass vessel (e.g., a Rotovap round-bottomed flask). Upon suspension in an aqueous solvent, the amphipathic lipid component molecules self-assemble into homogenous lipid vesicles. These lipid vesicles may subsequently be processed to have a selected mean diameter of

uniform size prior to complexing with, for example, pDNA according to methods known to those skilled in the art. For example, the sonication of a lipid solution is described in Felgner *et al., Proc. Natl. Acad. Sci. USA* 84,7413-7417 (1987) and in U.S. Pat. No. 5,264,618, the disclosures of which are incorporated herein by reference.

In the embodiments where the pharmaceutical composition includes a cationic lipid, the nucleic acid molecule construct(s) are complexed with lipids by mixing, for example, a pDNA solution and a solution of cationic lipid:co-lipid liposomes. Preferably, the concentration of each of the constituent solutions is adjusted prior to mixing such that the desired final pDNA/cationic lipid:co-lipid ratio and the desired pDNA final concentration will be obtained upon mixing the two solutions. For example, if the desired final solution is to be physiological saline (0.9% weight/volume), both pDNA and cationic lipid:co-lipid liposomes are prepared in 0.9% saline and then simply mixed to afford the desired complex. The cationic lipid:co-lipid liposomes are preferably prepared by hydrating a thin film of the mixed lipid materials in an appropriate volume of aqueous solvent by vortex mixing at ambient temperatures for about 1 minute. The thin films are prepared by admixing chloroform solutions of the individual components to afford a desired molar solute ratio followed by aliquoting the desired volume of the solutions into a suitable container. The solvent is removed by evaporation, first with a stream of dry, inert gas (e.g. argon) followed by high vacuum treatment.

Other hydrophobic and amphiphilic additives, such as, for example, sterols, fatty acids, gangliosides, glycolipids, lipopeptides, liposaccharides, neobees, niosomes, prostaglandins and sphingolipids, may also be included in the immunogenic compositions of the present invention. In such compositions, these additives may be included in an amount between about 0.1 mol % and about 99.9 mol % (relative to total lipid). Preferably, these additives comprise about 1-50 mol % and, most preferably, about 2-25 mol %. Preferred additives include lipopeptides, liposaccharides and steroids.

Nucleic acid molecules and/or polynucleotides of the present invention, e.g., pDNA, mRNA, linear DNA or oligonucleotides, may be solubilized in any of various buffers. Suitable buffers include, for example, phosphate buffered saline (PBS), normal saline, Tris buffer, and sodium phosphate (150 mM preferred). Insoluble polynucleotides may be solubilized in a weak acid or weak base, and then diluted to the desired volume with a buffer. The pH of the buffer may be adjusted as appropriate. In addition, a pharmaceutically acceptable additive can be used to provide an appropriate osmolarity. Such additives are within the purview of one skilled in the art.

The choice of polynucleotide form depends in part on the desired kinetics and duration of expression. When long-term delivery of the protein encoded by the polynucleotide is desired, the preferred form is DNA. Alternatively, when short-term transgene protein delivery is desired, the preferred form is mRNA, since RNA is rapidly translated into polypeptide, but is degraded more quickly than DNA.

In one embodiment, the polynucleotides of the present invention is RNA. Preferably, in this embodiment, the RNA is in the form of messenger RNA (mRNA). Methods for introducing RNA sequences into mammalian cells is described in U.S. Patent No. 5,580,859, the disclosure of which is incorporated herein by reference. A viral alphavector, a non-infectious vector useful for administering RNA, may be used to introduce RNA into mammalian cells. Methods for the *in vivo* introduction of alphaviral vectors to mammalian tissues are described in Altman-Hamamdzic, S., *et al.*, *Gene Therapy* 4, 815-822 (1997), the disclosure of which is incorporated herein by reference. Viral replicons, i.e., non-infectious RNA vectors packaged in a viral coat, e.g., a picornavirus coat or an alphavirus coat, are also useful for efficient administration of RNA. See, e.g., US Patent No. 5,766,602, U.S. Patent No. 5,614,413, and PCT Publication No. WO 95/07994, the disclosures of which are incorporated herein by reference.

Preferably, the polynucleotide is DNA. In the case of DNA, a promoter is preferably operably associated with the polynucleotide. The promoter may be

a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be included with the polynucleotide to direct cell-specific transcription of the DNA. An operable association is when a polynucleotide encoding molecule is associated to one or more regulatory sequences in such a way as to place expression of the molecule under the influence or control of the regulatory sequence(s). Two DNA sequences (such as a coding sequence and a promoter region sequence associated with the 5' end of the coding sequence) are operably associated if induction of promoter function results in the transcription of mRNA encoding for the desired antigen and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the expression regulatory sequences to direct the expression of the antigen, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a DNA sequence if the promoter was capable of effecting transcription of that DNA sequence. A variety of transcription control regions are known to those skilled in the art. Preferred transcription control regions include those which function in vertebrate cells, such as, but not limited to, promoter and enhancer sequences from cytomegaloviruses (preferably the intermediate early promoter, preferably in conjunction with intron-A), simian virus 40 (preferably the early promoter), retroviruses (such as Rous sarcoma virus), and picornaviruses (particularly an internal ribosome entry site, or IRES, enhancer region, also referred to herein as a CITE sequence). Other preferred transcription control regions include those derived from mammalian genes such as actin, heat shock protein, bovine growth hormone transcription control regions, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins).

Preferably, the polynucleotides of the present invention is part of a circular or linearized plasmid containing a non-infectious (i.e., does not infect vertebrate cells), nonintegrating (i.e., does not integrate into the genome of vertebrate cells) polynucleotide. A linearized plasmid is a plasmid that was previously circular but has been linearized, for example, by digestion with a restriction endonuclease. The polynucleotides of the present invention may comprise a sequence which directs the secretion of a polypeptide.

Polynucleotides of the present invention, e.g., pDNA, mRNA, linear DNA or oligonucleotides, may be solubilized in any of various buffers. Suitable buffers include, for example, phosphate buffered saline (PBS), normal saline, Tris buffer, and sodium phosphate (150 mM preferred). Insoluble polynucleotides may be solubilized in a weak acid or weak base, and then diluted to the desired volume with a buffer. The pH of the buffer may be adjusted as appropriate. In addition, a pharmaceutically acceptable additive can be used to provide an appropriate osmolarity. Such additives are within the purview of one skilled in the art.

In certain embodiments, a single antigen- or cytokine-encoding polynucleotide encoding one or more antigens or cytokines can be administered, either simultaneously or sequentially, with a polynucleotide expressing the pFlt-3 ligand or variant or fragment thereof as described herein. Alternatively, multiple antigen- or cytokine-encoding polynucleotides each encoding for one or more antigens or cytokines can be co-administered, either simultaneously or sequentially, with a polynucleotide expressing the pFlt-3 ligand or variant or fragment thereof as described herein. Methods of making various nucleic acid molecule constructs comprising antigen- or cytokine-encoding polynucleotides are well known to those skilled in the art of molecular biology, and disclosed, for example, in U.S. Patent Nos. 4,713,339 and 4,965,196, the disclosures of which are incorporated herein by reference.

The immunogenic compositions of the present invention may be administered according to any of various methods known in the art. For example,

U.S. Patent No. 5,676,954 reports on the injection of genetic material, complexed with cationic lipid carriers, into mice. Also, U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and PCT international patent application PCT/US94/06069 (WO 94/29469), the disclosures of which are incorporated herein by reference, provide methods for delivering DNA-cationic lipid complexes to mammals.

More specifically, the immunogenic compositions of the present invention may be administered to any tissue of a vertebrate, including, but not limited to, muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, tongue and connective tissue. Preferably, the compositions are administered to muscle, either skeletal or cardiac. Most preferably, the composition is administered to skeletal muscle.

Preferably, the immunogenic compositions of the present invention are administered by intramuscular (i.m.) or subcutaneous (s.c.) routes. Other suitable routes of administration include transdermal, intranasal, inhalation, transmucosal (i.e., across a mucous membrane), intra-cavity (e.g., oral, vaginal, or rectal), and intravenous (i.v.) administration.

Any mode of administration can be used so long as the mode results in the expression of the Flt-3 ligand and one or more antigenic molecules in an amount sufficient to elicit a measurable immune response in a vertebrate. This includes needle injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns" or pneumatic "needleless" injectors -- for example, Med-E-Jet (Vahlsing, H., *et al., J. Immunol. Methods* 171,11-22 (1994)), Pigjet (Schrijver, R., *et al., Vaccine* 15, 1908-1916 (1997)), Biojector (Davis, H., *et al., Vaccine* 12, 1503-1509 (1994); Gramzinski, R., *et al., Mol. Med.* 4, 109-118 (1998)), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. The preferred mode of administration is injection.

Determining an effective amount of an immunogenic composition depends upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the subject, the precise condition requiring treatment and its severity, and the route of administration. Based on the above factors, determining the precise amount, number of doses, and timing of doses are within the ordinary skill in the art and will be readily determined by the attending physician or veterinarian.

In certain embodiments, the immunogenic composition is administered as a pharmaceutical composition. Such a pharmaceutical composition can be formulated according to known methods, whereby the substance to be delivered is combined with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their preparation are described, for example, in Remington's Pharmaceutical Sciences, 16th Edition, A. Osol, ed., Mack Publishing Co., Easton, PA (1980), and Remington's Pharmaceutical Sciences, 19th Edition, A.R. Gennaro, ed., Mack Publishing Co., Easton, PA (1995). The pharmaceutical composition can be formulated as an emulsion, gel, solution, suspension, lyophilized form, or any other form known in the art. In addition, the pharmaceutical composition can also contain pharmaceutically acceptable additives including, for example, diluents, binders, stabilizers, and preservatives.

Administration of pharmaceutically acceptable salts of the nucleic acid molecule constructs described herein is preferred. Such salts can be prepared from pharmaceutically acceptable non-toxic bases including organic bases and inorganic bases. Salts derived from inorganic bases include sodium, potassium, lithium, ammonium, calcium, magnesium, and the like. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, basic amino acids, and the like.

For aqueous pharmaceutical compositions used *in vivo*, use of sterile pyrogen-free water is preferred. Such formulations will contain an effective amount of the immunogenic composition together with a suitable amount of

vehicle in order to prepare pharmaceutically acceptable compositions suitable for administration to a vertebrate.

The present invention can be used in connection with the prophylactic and/or therapeutic treatment of vertebrate infections arising from pathogens of various species, by administering into the tissues of the vertebrate a polynucleotide encoding a suitable antigen from a given pathogen, in combination with a polynucleotide encoding a Flt-3 ligand, or an active variant, or fragment thereof as described herein. Examples of such antigens include, but are not limited to bacterial antigens, viral antigens, parasite antigens, and fungal antigens. Examples of viral antigens include, but are not limited to, adenovirus antigens, alphavirus antigens, calicivirus antigens, e.g., a calicivirus capsid antigen, coronavirus antigens, distemper virus antigens, Ebola virus antigens, enterovirus antigens, flavivirus antigens, hepatitis virus (A-E) antigens, e.g., a hepatitis B core or surface antigen, herpesvirus antigens, e.g., a herpes simplex virus or varicella zoster virus glycoprotein antigen, immunodeficiency virus antigens, e.g., a human immunodeficiency virus envelope or protease antigen, infectious peritonitis virus antigens, influenza virus antigens, e.g., an influenza A hemagglutinin or neuraminidase antigen, leukemia virus antigens, Marburg virus antigens, oncogenic virus antigens, orthomyxovirus antigens, papilloma virus antigens, parainfluenza virus antigens, e.g., hemagglutinin/neuraminidase antigens, paramyxovirus antigens, parvovirus antigens, pestivirus antigens, picorna virus antigens, e.g., a poliovirus capsid antigen, pox virus antigens, e.g., a vaccinia virus antigen, rabies virus antigens, e.g., a rabies virus glycoprotein G antigen, reovirus antigens, retrovirus antigens, rotavirus antigens, as well as other cancer-causing or cancer-related virus antigens. Examples of bacterial antigens include, but are not limited to, *Actinomyces,* antigens *Bacillus* antigens, *Bacteroides* antigens, *Bordetella* antigens, *Bartonella* antigens, *Borrelia* antigens, *e.g.,* a *B. bergdorferi Osp*A antigen, *Brucella* antigens, *Campylobacter* antigens, *Capnocytophaga* antigens, *Chlamydia* antigens, *Clostridium* antigens, *Corynebacterium* antigens, *Coxiella* antigens, *Dermatophilus* antigens,

Enterococcus antigens, *Ehrlichia* antigens, *Escherichia* antigens, *Francisella* antigens, *Fusobacterium* antigens, *Haemobartonella* antigens, *Haemophilus* antigens, e.g., *H. influenzae* type b outer membrane protein antigens, *Helicobacter* antigens, *Klebsiella* antigens, L-form bacteria antigens, *Leptospira* antigens, *Listeria* antigens, *Mycobacteria* antigens, *Mycoplasma* antigens, *Neisseria* antigens, *Neorickettsia* antigens, *Nocardia* antigens, *Pasteurella* antigens, *Peptococcus* antigens, *Peptostreptococcus* antigens, *Pneumococcus* antigens, *Proteus* antigens, *Pseudomonas* antigens, *Rickettsia* antigens, *Rochalimaea* antigens, *Salmonella* antigens, *Shigella* antigens, *Staphylococcus* antigens, *Streptococcus* antigens, e.g., *S. pyogenes* M protein antigens, *Treponema* antigens, and *Yersinia* antigens, e.g., *Y pestis* F1 and V antigens.

Examples of fungal antigens include, but are not limited to, *Absidia* antigens, *Acremonium* antigens, *Alternaria* antigens, *Aspergillus* antigens, *Basidiobolus* antigens, *Bipolaris* antigens, *Blastomyces* antigens, *Candida* antigens, *Coccidioides* antigens, *Conidiobolus* antigens, *Cryptococcus* antigens, *Curvalaria* antigens, *Epidermophyton* antigens, *Exophiala* antigens, *Geotrichum* antigens, *Histoplasma* antigens, *Madurella* antigens, *Malassezia* antigens, *Microsporum* antigens, *Moniliella* antigens, *Mortierella* antigens, *Mucor* antigens, *Paecilomyces* antigens, *Penicillium* antigens, *Phialemonium* antigens, *Phialophora* antigens, *Prototheca* antigens, *Pseudallescheria* antigens, *Pseudomicrodochium* antigens, *Pythium* antigens, *Rhinosporidium* antigens, *Rhizopus* antigens, *Scolecobasidium* antigens, *Sporothrix* antigens, *Stemphylium* antigens, *Trichophyton* antigens, *Trichosporon* antigens, and *Xylohypha* antigens.

Examples of protozoan parasite antigens include, but are not limited to, *Babesia* antigens, *Balantidium* antigens, *Besnoitia* antigens, *Cryptosporidium* antigens, *Eimeri* antigens *a* antigens, *Encephalitozoon* antigens, *Entamoeba* antigens, *Giardia* antigens, *Hammondia* antigens, *Hepatozoon* antigens, *Isospora* antigens, *Leishmania* antigens, *Microsporidia* antigens, *Neospora* antigens, *Nosema* antigens, *Pentatrichomonas* antigens, *Plasmodium* antigens, e.g., *P. falciparum* circumsporozoite (PfCSP), sporozoite surface protein 2 (PfSSP2),

carboxyl terminus of liver state antigen 1 (PfLSA-1 c-term), and exported protein 1 (PfExp-1) antigens, *Pneumocystis* antigens, *Sarcocystis* antigens, *Schistosoma* antigens, *Theileria* antigens, *Toxoplasma* antigens, and *Trypanosoma* antigens.

Examples of helminth parasite antigens include, but are not limited to, *Acanthocheilonema* antigens, *Aelurostrongylus* antigens, *Ancylostoma* antigens, *Angiostrongylus* antigens, *Ascaris* antigens, *Brugia* antigens, *Bunostomum* antigens, *Capillaria* antigens, *Chabertia* antigens, *Cooperia* antigens, *Crenosoma* antigens, *Dictyocaulus* antigens, *Dioctophyme* antigens, *Dipetalonema* antigens, *Diphyllobothrium* antigens, *Diplydium* antigens, *Dirofilaria* antigens, *Dracunculus* antigens, *Enterobius* antigens, *Filaroides*,antigens *Haemonchus* antigens, *Lagochilascaris* antigens, *Loa* antigens, *Mansonella* antigens, *Muellerius* antigens, *Nanophyetus* antigens, *Necator* antigens, *Nematodirus* antigens, *Oesophagostomum* antigens, *Onchocerca* antigens, *Opisthorchis* antigens, *Ostertagia* antigens, *Parafilaria* antigens, *Paragonimus* antigens, *Parascaris* antigens, *Physaloptera* antigens, *Protostrongylus* antigens, *Setaria* antigens, *Spirocerca*,antigens *Spirometra* antigens, *Stephanofilaria* antigens, *Strongyloides* antigens, *Strongylus* antigens, *Thelazia* antigens, *Toxascaris* antigens, *Toxocara* antigens, *Trichinella* antigens, *Trichostrongylus* antigens, *Trichuris* antigens. *Uncinaria* antigens, and *Wuchereria* antigens.

Examples of ectoparasite antigens include, but are not limited to, antigens (including protective antigens as well as allergens) from fleas; ticks, including hard ticks and soft ticks; flies, such as midges, mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, stable flies, myiasis-causing flies and biting gnats; ants; spiders, lice; mites; and true bugs, such as bed bugs and kissing bugs.

Examples of tumor-associated antigens include, but are not limited to, a tumor-specific immunoglobulin variable region, a GM2 antigen, a Tn antigen, an sTn antigen, a Thompson-Friedenreich antigen (TF), a Globo H antigen, an Le(y) antigen, a MUC1 antigen, a MUC2 antigen, a MUC3 antigen, a MUC4 antigen, a MUC5AC antigen, a MUC5B antigen, a MUC7 antigen, a carcinoembryonic

antigen, a beta chain of human chorionic gonadotropin (hCG beta) antigen, a HER2/neu antigen, a PSMA antigen, a EGFRvIII antigen, a KSA antigen, a PSA antigen, a PSCA antigen, a GP100 antigen, a MAGE 1 antigen, a MAGE 2 antigen, a TRP 1 antigen, a TRP 2 antigen, and a tyrosinase antigen.

Also included in the present invention are fragments or variants of the foregoing antigens, and any combination of the foregoing antigens. Additional antigens may be found, for example in "Foundations in Microbiology," Talaro, *et al.*, eds., McGraw-Hill Companies (Oct.,1998), Fields, *et al.*, "Virology," 3d ed., Lippincott-Raven (1996), "Biochemistry and Molecular Biology of Parasites," Marr, et al., eds., Academic Press (1995), and Deacon, J., "Modern Mycology," Blackwell Science Inc (1997), which are incorporated herein by reference.

In a preferred embodiment, the present invention can be applied as a prophylactic and/or therapeutic treatment of vertebral diseases associated with parasites such as malaria. Preferred antigens encoded for such application includes pre-erythrocytic antigens from the human malaria parasite *Plasmodium falciparum* including circumsporozoite protein (PfCSP), sporozoite surface protein 2 (PfSSP2), carboxyl terminus of liver stage antigen 1 (PfLSA-1 c-term), exported protein 1 (PfExp-1), any fragment thereof, and any combination thereof.

In another preferred embodiment, the present invention can be applied as a prophylactic and/or therapeutic treatment for Aujeszky's disease (AD) in pigs caused by the alphaherpesvirus pseudorabies virus (PrV), also designated as *Suid Herpesvirus 1*.

In yet another preferred embodiment, the present invention can be applied as a prophylactic and/or therapeutic treatment for hepatitis B and C in human patients caused by hepatitis B virus (HBV) and hepatitis C virus (HCV).

The present invention can also be applied in immunologically-based therapeutic and/or prophylactic treatments of cancer including, but not limited to, cancers of oral cavity and pharynx (i.e., tongue, mouth, pharynx), digestive system (i.e., esophagus, stomach, small intestine, colon, rectum, anus, anal canal,

anorectum, liver, gallbladder, pancreas), respiratory system (i.e., larynx, lung), bones, joints, soft tissues (including heart), skin, melanoma, breast, reproductive organs (i.e., cervix, endometirum, ovary, vulva, vagina, prostate, testis, penis), urinary system (i.e., urinary bladder, kidney, ureter, and other urinary organs), eye, brain, endocrine system (i.e., thyroid and other endocrine), lymphoma (i.e., B-cell lymphoma including non-hodgkin's lymphoma , hodgkin's disease), multiple myeloma, leukemia (i.e., acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia). Antigens to include in an immunogenic composition of the present invention are described, e.g., in Chamberlain, *Drugs* 57(3):309-25 (March, 1999), Nawrocki and Mackiewicz, *Cancer Treat Rev* 25(1):29-46 (Feb.,1999), Rosenberg, *Immunity* 10(3):281-7 (Mar., 1999), Rosenberg *et al., Adv Caner Res* 70:145-77 (1996), Pandey *et al., Eur J Surg Oncol* 25(2):209-14 (Apr., 1999), Herlyn *et al., Ann Med.* 31(1):66-78 (Feg., 1999), and Rosenberg, *Immunol Today* 18(4):175-82 (Apr., 1997), which are incorporated herein by reference.

In a preferred embodiment the present invention can be applied as a therapeutic treatment of B-cell lymphoma through the use of a polynucleotide encoding an antigen comprising a variable region of an immunoglobulin expressed by the B-cell lymphoma containing tumor-specific idiotype determinants and a constant region.

An additional embodiment of the present invention is directed to combining any of the cancer treatment methods of the present invention with one or more additional cancer therapies including, but not limited to bone marrow transplant, cord blood cell transplant, surgery, chemotherapy, radiation therapy, and immunotherapy. The polynucleotide, nucleic acid molecule construct(s), immunogenic composition or pharmaceutical composition of the present invention can be administered prior to the commencement of one or more of the additional cancer therapies, during the practice of one or more of the additional cancer therapies, and after the end of one or more of the additional cancer therapies.

Types of bone marrow transplant include, but are not limited to autologous bone marrow transplant and heterologous (*i.e.*, from a donor) bone marrow transplant.

Types of surgery include, but are not limited to surgery for breast cancer, prostate cancer, colon cancer, brain cancer, and head and neck cancer.

Chemotherapeutic agents include, but are not limited to alkylating agents, including mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, dicarbazine, streptazocine, carmustine, lomustine, semustine, chlorozotocin, busulfan, triethylenemelamine, thiotepa, hexamethylmelamine; antimetabolites, including methotrexate; pyrimidine analogs, including fluorouracil, 5-fluorouracil, floxuridine (5'-fluoro-2'-deoxyuridine), idoxuridine, cytarabine, N-phosphonoacetyl-L-aspartate, 5-azacytidine, azaribine, 6-azauridine, pyrazofuran, 3-deazauridine, acivicin; purine analogs, including thioguanine, mercaptopurine, azathioprine, pentostatin, erythrohydroxynonyladenine; vinca alkaloids, including vincristine and vinblastine; epipodophyllotoxins, including etoposide and teniposide; antibiotics, including dactinomycin, daunorubicin, doxorubicin, bleomycin sulfate, plicamycin, mitomycin; enzymes, including L-asparaginase; platinum coordination complexes, including cisplatin, carboplatin; hydroxyurea, procarbazine, mitotane; and hormones or related agents, including adrenocorticosteroids such as prednisone and prednisolone; aminoglutethimide; progestins such as hydroxyprogesterone caproate, medroxyprogesterone acetate, megesterol acetate, estrogens and androgens such as diethylstilbestrol, fluoxymesterone, ethynyl estradiol, antiestrogens such as tamoxifen, and gonadotropin-releasing hormone analogs such as leuprolide.

The present invention may also be applied in the therapeutic and/or prophylactic treatment of autoimmune diseases such as rheumatoid arthritis and osteoarthritis.

The present invention also provides kits for use in immunotherapy comprising an administration means and a container means containing a Flt-3

ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides in a sterile environment. Also provided are kits for use in immunotherapy comprising an administration means and a container means containing a Flt-3 ligand-encoding polynucleotide, one or more antigen- or cytokine-encoding polynucleotides, and one or more cationic lipid compounds in a sterile environment. Examples of cationic lipid compounds are described above. The nucleic acid molecule constructs comprising a Flt-3 ligand-encoding polynucleotide, one or more antigen- or cytokine-encoding polynucleotides and the cationic lipid compounds may be in the same container means or in separate container means. Preferably, the polynucleotides are in the amount of 1 ng to 30 mg.

Container means include glass containers, plastic containers, or strips of plastic or paper. In one embodiment, the container means is a syringe and the administration means is a plunger. In another embodiment, the administration means is a catheter. The construct can be in the form of a pharmaceutical composition and can contain a pharmaceutically acceptable carrier. Pharmaceutical compositions are described above. The kit can further comprise a pharmaceutically acceptable carrier in a separate container means.

The kit can further comprise an instruction sheet for administration of the composition into a mammal. The polynucleotide components of the pharmaceutical composition are preferably provided as a liquid solution, such as a suspension, a solution, or an emulsion; or in lyophilized form as a dried powder or a cake. If the nucleic acid molecule construct(s) comprising a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides are provided in lyophilized form, preferably the kit further comprises a container means containing a suitable vehicle, such as sterile pyrogen-free water, for reconstitution of the lyophilized nucleic acid molecule construct(s) comprising a Flt-3 ligand-encoding polynucleotide and one or more antigen- or cytokine-encoding polynucleotides, or any buffer described herein, including PBS, normal saline, Tris buffer, and sodium phosphate vehicle.

The container in which the pharmaceutical composition is packaged prior to use can comprise a hermetically sealed container enclosing an amount of the lyophilized formulation or a solution containing the formulation suitable for a pharmaceutically effective dose thereof, or multiples of an effective dose. The pharmaceutical composition is packaged in a sterile container, and the hermetically sealed container is designed to preserve sterility of the pharmaceutical formulation until use. Optionally, the container can be associated with administration means and or instruction for use.

Having now generally described the invention, the same will become more readily understood by reference to the following specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

Described herein are: 1) the prophylactic application of a Flt-3 ligand-encoding polynucleotide in combination with a tumor specific antigen-encoding polynucleotide in a murine system; 2) a treatment regimen for using a Flt-3 ligand-encoding polynucleotide in combination with a tumor specific antigen-encoding polynucleotide in a humans; 3) prophylactic application of a Flt-3 ligand-encoding polynucleotide in combination with a malarial antigen-encoding polynucleotide in humans; 4) prophylactic application of a Flt-3 ligand-encoding polynucleotide in combination with a hepatitis B antigen-encoding polynucleotide; and 5) a treatment regimen with a Flt-3 ligand-encoding polynucleotide in combination with a hepatitis B or C antigen encoding polynucleotide for treatment of patients with chronic hepatitis.

Example 1

Prophylactic Application of Flt-3 Ligand-encoding pDNA in Combination with Tumor-specific Antigen-encoding pDNA in Murine System

The purpose of the present example is to demonstrate the ability of the present invention to enhance protection from tumor challenge in murine B-cell lymphoma model.

The 38C13 murine B-cell lymphoma tumor cell line displays a surface immunoglobulin with a unique idiotype (Id). This idiotype is used as a tumor-specific antigen in immunization protocols designed to test various vaccine strategies. However, an anti-idiotypic antibody response may be necessary but is not sufficient to protect vaccinated mice from tumor challenge (Sternas LA *et al., Cancer Treat. Res.* 99:267-273(1999); Kwak LW *et al., Proc. Natl. Acad. Sci. U S A* 93:10972-10977 (1996); King CA *et al., Nat. Med.* 4:1281-1286 (1998); Haimovich J *et al., Cancer Immunol. Immunother.* 47:330-336 (1999). Also, the generation of an anti-Id CTL response correlates with a better outcome in human non-Hodgkin's lymphoma patients (Nelson EL *et al., Blood* 88:580-589 (1996)), and antibody depletion studies have shown that both CD4 and CD8 cellular responses are required for protection (Kwak LW *et al., Proc. Natl. Acad. Sci. U S A* 93:10972-10977 (1996); Biragyn A *et al., Nat. Biotechnol.* 17:253-258 (1999).

The experimental vaccine protocol of the present example utilizes a human/mouse chimeric immunoglobulin DNA construct (pId). The light and heavy chain variable regions of the 38C13 immunoglobulin cDNA are fused in-frame with the respective xenogeneic human kappa and gamma constant region sequences. The human constant region portion contains T helper epitopes that stimulate a robust CD4 response that is required for protection. The vector used in the present invention, VR-1623, comprises a bicistronic CITE sequence which allows both chains of the immunoglobulin molecule to be expressed from a single

RNA transcript. This plasmid is co-injected with a Flt-3 ligand-encoding pDNA to demonstrate the immunogenicity enhancement activity of the pFlt-3 ligand on pId.

Preparation of pDNAs

VR6200 expresses a secreted form of murine Flt-3 ligand. Total bone marrow RNA was isolated from a single C57Bl/6 mouse using a Qiagen Rneasy Mini Kit (Valencia, CA, catalog # 74103) according to the manufacturer's protocol. Briefly, the bone marrow cells were flushed from the femurs with sterile PBS using a 28 gauge needle. The bone marrow cells were pelleted by centrifugation, resuspended in Qiagen lysis buffer, and the protocol followed. Approximately 150 μg of total RNA was obtained.

First strand cDNA was synthesized from 5 μg of the bone marrow total RNA using the SuperScript Preamplification System (Life Technologies, Inc., Gaithersburg, MD. catalog# 18089-011). Two μl of bone marrow cDNA was used in a 50 μl PCR reaction using native *Pfu* polymerase from Stratagene (La Jolla, CA. catalog # 600136) with the supplied buffer. The forward PCR primer CAC GAA TTC GCC GCC ACC ATG ACA GTG CTG GCG CCA (SEQ ID NO:25) and the reverse primer GCC GCT AGC TCA CTG CCT GGG CCG AGG CTC T (SEQ ID NO:26) were synthesized by Sigma-Genosys (The Woodlands, TX). The forward primer codes for an *Eco*RI restriction site followed by a consensus Kozak sequence followed by the Flt-3 ligand ATG start site and coding region. The reverse primer codes for an *Nhe*I restriction site, and places a stop codon just 5' of the transmembrane region. The final cDNA construct contains nucleotides 32 through 598 of the Flt-3 ligand cDNA (GenBank accession # L23636, denoted herein as SEQ ID NO:7). The construct codes for a secreted form of murine Flt-3 ligand lacking the transmembrane and cytoplasmic domains, and having the amino acid sequence denoted herein as SEQ ID NO:9. This form of murine Flt-3 ligand has been shown to be biologically active. (McClanahan, *et al., ibid.*)

The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The PCR product was digested with *Eco*RI and *Nhe*I and cloned into the same sites of the plasmid VR-1051 (Norman *et al., Vaccine* 1997 Jun;15(8):801-3) using standard molecular biology techniques. Individual clones were then sequenced (Retrogen Inc., San Diego, CA), and a perfect clone was named VR6200 (SEQ ID NO:6).

VR1605 is a generic cloning vector containing the constant regions of the human kappa light chain and gamma 1 heavy chain separated by a CITE (cap independent translational enhancer) sequence. VR1605 was constructed in three steps. (1) The CITE sequence was PCR amplified from the vector pCITE (Novagen, Madison, WI) using the primers 5'- AAA ACT GCA GGC CTT GCT GGC CAC GCG TTA TTT TCC ACC ATA TT -3' (SEQ ID NO:27) and 5'- CGC GGA TCC GGC CGC TGC GGC CAT GGT ATT ATC ATC -3'(SEQ ID NO:28). The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The 535-bp CITE fragment was cloned into the *Pst*I – *Bam*HI sites of VR1012 (Norman *et al.*, *Vaccine* Jun;15(8):801-3 (1997)) to create VR1031. (2) The human Kappa constant region was PCR amplified from peripheral blood cDNA (prepared as in the Flt-3 ligand cDNA example above) using the primer set 5'- CGA CGC GTG TGG CTG CAC CAT CTG TCT GGC CCT TCC GGC CAT CTG ATG AGC AGT TG -3' (SEQ ID NO:29) and 5'- CGA CGC GTT CAA CAC TCT CCC CTG TTG AAG CT -3' (SEQ ID NO:30). The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The 320-bp fragment was cloned into VR1031 as an *Mlu*I fragment to create VR1603. (3) The human Gamma 1 constant region was PCR amplified from peripheral blood cDNA using the primer set 5'- GGA AGA TCT ACC AAG GGC CCA TCG GGC CTC TCC CCC TGG CAC CCT CC -3' (SEQ ID NO:31) and 5'- GGA AGA TCT CAG GAT CTC GGA GAC AGG GAG AGG CT -3' (SEQ ID NO:32). The PCR

cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The 980-bp fragment was cut with *Bgl*II and *Bam*HI and cloned into the *Bam*HI site of VR1603 to create VR1605 (SEQ ID NO:2). This vector contains the human kappa and gamma 1 constant regions but no variable regions; therefore it does not express a functional protein. It serves as a negative control for the vaccination studies.

VR1623 is a bicistronic chimeric Id vector. For its construction, murine variable region cDNAs were cloned into VR1605 to have a chimeric heavy and light chain surface immunoglobulin expressed from a single mRNA transcript. The variable (V) heavy and light chain segments of the immunoglobulin expressed by 38C13 cells were PCR amplified from pId obtained from Ronald Levy (Syrengelas, *et al., Nat. Med.*, 2:1038-1041 (1996)).

The murine V kappa segment was PCR amplified with the primer set 5'- CTG CAG AAG GCC TTG CTG GCC GCC ATG GGA CCG TCT GTT CAG TTC -3' (SEQ ID NO:33) and 5'- TGG TGC GGC CAC CGT GGC CTT TAT TTC CAA CTT G –3' (SEQ ID NO:34). The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

The murine V heavy segment was PCR amplified with the primer set 5'- CTG CAG AAG GCC GCA GCG GCC GAG TTG TGG CTG AAC TGG ATT TTC -3' (SEQ ID NO:35) and 5'- CCG ATG GGC CCT TGG GGC CAG CTG AGG AGA CGG T -3' (SEQ ID NO:36). The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

The 38C13 light chains were cloned into VR1605 in a single step as unique *Sfi*I fragments due to the unique five-bp N internal sequence of each *Sfi*I site, to create plasmid VR1623 (SEQ ID NO:1). The kappa variable segment includes nucleotides 1497 to 1890 of the vector VR1623, and the heavy chain variable region includes nucleotides 2723 to 3171 of the vector VR1623.

Plasmid DNA was transformed into *Escherichia coli* DH10B-competent cells, and a single colony was grown in Terrific Broth (Sambrook, *et al.*, *ibid.*, p. A.2 (1989)) complemented with 30 mg/ml kanamycin in a 2.8 L Fernbach flask at 37°C for approximately 20 hours.

Large scale plasmid isolation was carried out using the procedure and reagents supplied by the Qiagen EndoFree Plasmid Giga kit (catalog # 12391). The DNA was precipitated with isopropanol and resuspended in 150 mM Na_2PO_4, pH 7. The concentration was determined by UV spectroscopy.

FLT-3 Ligand in Vitro Expression Demonstrated by Western Blot Analysis

Expression of Flt-3 ligand protein from plasmid VR6200 was demonstrated in eukaryotic cells by the following method. UM449 cells (kindly provided by Mark Cameraon and Dr. Gary Nabel at the University of Michigan) were transfected with 5 μg of VR6200 or VR1051, formulated with DMRIE/DOPE (2:1 lipid/DNA molar ratio). Forty eight hours later, culture media was collected and the transfected cells were lysed in 250 μl of 1X cell lysis buffer (Promega, Madison WI, catalog # E153A). The supernatant and cell lysate were assayed for Flt-3 ligand expression by Western blot analysis using standard procedures.

Briefly, 20 μl of supernatant and cell lysate was added to an equal volume of sample buffer (NOVEX, San Diego, CA, catalog # LC3675), boiled for 10 minutes and loaded on a 4-20% Tris-Glycine SDS polyacrylamide gel (NOVEX, catalog # EC6025) and electrophoresed according to the manufacturer's instructions. The gel was blotted onto Immobilon-P membrane (Millipore Corp., Bedford, MA, Fisher catalog # IPUH-000-10) and hybridized with polyclonal anti-mouse Flt-3 ligand antiserum (R & D Systems, Inc., Minneapolis, MN, catalog # AF427). The antiserum was diluted 1:1000 in BBS (1X BBS: 200 mM Boric acid, 90 mM NaCl, pH8.3) before use. The secondary antibody used for detection was rabbit anti-Goat IgG Alkaline Phosphatase (1:1000 dilution in BBS) (Jackson ImmunoResearch Laboratories, Inc., Bar Harbor, ME, catalog #305-055-088).

The Western blot showed three bands of molecular weight between 20K-32K daltons reflecting the multiple glycosylation products of Flt-3 ligand (McClanahan *et al., Blood* 1996 Nov 1;88(9):3371-82).

Cell lines and tumor models

The 38C13 B cell lymphoma line (Bergman *et al., Eur. J. Immunol.* 7:413-417(1977) was obtained from Dr. Ron Levy at Stanford University. A working cell bank of passage # 4 38C13 cells was established and maintained at –80°C using standard tissue culture methods. For the experimental tumor cell challenge, 38C13 cells were grown for 48 hours in RPMI 1640, 10% heat-inactivated FCS, 2mM L-glutamine, 100U/ml penicillin, 100μg/ml streptomycin, and 50 μM β-ME at 37°C, 5% CO_2 in a humidified incubator. The cells were washed three times in RPMI, and diluted to 1,000 cells/ml. Mice were injected intraperitoneally (i.p.) with 500 cells (0.5ml) two weeks after the last DNA immunization.

Animal Immunizations

Animal care throughout the study was in compliance with the "Guide for the Use and Care of Laboratory Animals," U.S. Department of Health and Human Services, National Institutes of Health (NIH Publication No. 86-23, revised 1985). C3H/HeN female mice were ordered from Harlan Sprague Dawley Inc. (HSD, San Diego, CA) at 5 to 6 weeks of age. Groups of 10 mice each were used for each study. Each mouse received multiple injections of 100 µl (50 µl/leg) of a DNA mixture containing 100 µg of VR1623 or VR1605, and 100 µg of VR6200 or VR1051 in 150mM NaH_2PO_4 (pH7). Mice were immunized with DNA intramuscularly by bilateral injection in the rectus femoris. At least one week after the last DNA injection all mice were bled for serum. Two weeks after the last DNA injection, mice were challenged with 500 38C13 tumor cells injected intraperitoneally. All animals were monitored daily, and moribund animals were euthanized.

38C13 IgG Anti-Id Antibody Levels in Vaccinated Animals

Serum collected from the animals was tested for Anti-Id IgG antibody levels by serial dilution using a standard ELISA assay. Briefly, the levels of anti-Id IgG antibodies in mouse sera were determined utilizing 38C13 idiotype protein purified from ascites (38c131.2), by Southern Biotechnology Associates Inc. (Birmingham, AL). ELISA plates were coated with 38C13 (2 ug/ml) and incubated overnight at 4°C. The plates were then washed, blocked with 5% nonfat milk, and incubated with dilutions of the mouse sera for 2-4 hr at room temperature. The plates were then washed and incubated with alkaline phosphatase-conjugated goat anti-mouse Fc-specific IgG (Jackson ImmunoResearch Laboratories Inc., Bar Harbor, ME) for 2 hr at room temperature, washed again, and incubated with substrate (p-nitrophenylphosphate) for 30min at room temperature. O.D. readings were taken at 405 nm using an ELISA plate reader (Molecular Devices, Menlo Park, CA). Serum antibody levels were determined by comparing serum titration curves with

a standard curve obtained with a known concentration of an affinity-purified anti-Id monoclonal antibody (S1C5) (Southern Biotechnology Associates Inc. Birmingham, AL).

Immune Response Enhancement

As shown in Figures 7 and 8, soluble Flt-3 ligand greatly enhanced the immunogenicity of co-injected naked DNA encoding the idiotype antigen as measured by increased tumor protection and antibody titer. Three groups of mice were used in the study. One group (n=9) was co-injected with VR6200 and VR1623 (100 µg each) on days 0, 14, and 28, and challenged with $5x10^2$ 38C13 tumor cells two weeks following the last injection. control groups (n=10 each) were co-injected with VR1623 and VR1051, or VR1605 or alone (200µg) on days 0, 14, and 28, and challenged with $5x10^2$ 38C13 tumor cells two weeks following the last injection.

The co-injection of a Flt-3 ligand-encoding plasmid (100 µg of VR6200) with a tumor-specific antigen-encoding plasmid (100 µg of VR1623) significantly enhanced protection from tumor challenge (Figure 7). Eight out of nine mice injected with VR1623 and VR6200 survived the challenge as compared to zero out of ten mice surviving after being immunized with VR1623 and the control plasmid, VR1051. This increased survival was statistically significant p=0.00007. Furthermore, the co-injection of a Flt-3 ligand-encoding plasmid (VR6200) with an idiotype antigen-encoding plasmid (VR1623) resulted in greatly enhanced anti-Id antibody titer relative to mice injected with VR1623 and VR1051, or with VR1623 alone (Figure 8 and Table 1).

Table 1: Serum Anti-Id IgG ng/ml (prebleed subtracted)				
Animal #	PNegative VR1605	pld VR1623	pld+pBlank VR1623+1051	pld+pFlt-3 lig VR1623+6200
1	0	0	0	13359
2	0	17401	0	13333
3	0	0	0	24050
4	0	511	0	51577
5	0	2851	3090	2203
6	0	3500	0	9
7	0	0	0	22277
8	0	1539	0	25375
9	0	0	3040	527
10	0	0	2156	

pFlt3-ligand mode of action

Flt-3 ligand protein administration has been shown to have anti-tumor effects (Chen K *et al., Cancer Res.* 1997, 57:3511-3516; Lynch DH, *Crit. Rev. Immunol.* (1998) 18:99-107.). To test whether Flt-3 ligand protein encoded *in vivo* by a plasmid vector (i.e. VR6200) acts in a similar manner in tumor protection, the survival of mice vaccinated with pId (VR1623) and pFlt-3 ligand (VR6200) was compared with the survival of mice vaccinated with pNegative (VR1605) and pFlt-3 ligand (VR6200). Four groups of mice (n=10 per group) were injected on days 0 and 7 with either VR1623 or VR1605 in combination with either VR6200 or VR1051 (100 μg of each pDNA), and challenged with $5x10^2$ 38C13 tumor cells two weeks following the last injection.

As shown in Figure 9, pFlt-3 ligand increased the protection of mice only when co-injected with pId. Injection of pFlt-3ligand without pId (pNegative + pFlt-3ligand group) was as ineffective as injecting negative control plasmids i.e. no protection. This shows that Flt-3 ligand delivered via plasmid DNA, by itself, is not likely to produce systemic anti-tumor activity. Rather, the Flt-3 ligand plasmid acts to enhance the immune response to a co-injected plasmid encoding an antigen.

Example 2

Treatment Regimen Using DNA Vaccine Encoding Tumor-Specific Idiotype and Flt-3Ligand for Patients with B-Cell Lymphoma

To prevent relapse of B-cell lymphoma, patients with non-Hodgkin's B-cell lymphoma are recruited, and a lymph node biopsy is obtained from each patient to produce a custom Id DNA vaccine comprising (1) pDNA encoding human Flt-3 ligand; and (2) pDNA encoding immunoglobulins expressed by each patient's tumor; in a pharmaceutically acceptable vehicle according to the method described below. All patients then receive standard chemotherapy wherein the timing and choice are determined by their physician. The therapy is continued until the patients achieve maximal response. Immunizations are initiated approximately 2 months after the completion of the chemotherapy wherein the patients are in remission period. Patients are injected with 0.8 to 2.0 mg each of pFlt-3lig and pId on day 0 and then 2, 6, 10, and 20 weeks later in alternate deltoids with the DNA vaccine encoding their own tumor Ig protein as described above. Patients are observed for toxicity, immune responses, and tumor status using standard procedures described below for 6-7 years from diagnosis and 5-6 years from the last chemotherapy given before vaccine treatment.

<u>Preparation of pDNAs.</u>

VR6230 contains a secreted form of human Flt-3 ligand cDNA. Total RNA from human bone marrow and peripheral blood cells was made with the Qiagen Rneasy Mini Kit as described in Example 1, above. The forward PCR primer GCG GAA TTC GCC GCC ACC ATG ACA GTG CTG GCG CCA GC (SEQ ID NO:37) and the reverse primer GCG GCT AGC TCA AGG GGG CTG CGG GGC TG (SEQ ID NO:38) were synthesized by Sigma-Genosys (The Woodlands, TX), based on the sequence of a human Flt-3 ligand cDNA sequence (GenBank accession #NM 001459, SEQ ID NO:22). The forward primer codes for an *Eco*RI restriction site followed by a consensus Kozak sequence followed

by the Flt-3 ligand ATG start site and coding region. The reverse primer codes for an *Nhe*I restriction site followed by a stop codon and coding sequence just 5' of the transmembrane region. The final cDNA construct contains nucleotides 93 through 647 of the Flt-3 ligand cDNA, encoding a polypeptide having amino acid sequence SEQ ID NO:24. The construct codes for a secreted form of human Flt-3 ligand lacking the transmembrane and cytoplasmic domains.

The PCR cycling conditions were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute. The PCR product was digested with *Eco*RI and *Nhe*I and cloned into the same sites of the plasmid VR-1051 (Norman *et al., ibid.*) using standard molecular biology techniques. Individual clones were then sequenced (Retrogen Inc., San Diego, CA), and a perfect clone was named VR6230 (SEQ ID NO:39).

The vector VR-1632 was designed to serve as a generic bicistronic human vaccine vector that contains the murine kappa light chain and gamma 1 heavy chain constant regions. Human tumor-specific variable regions are cloned into VR-1632 to produce a chimeric immunoglobulin analogous to the chimeric murine vaccine VR-1623. The tumor-specific light and heavy chain variable regions will be cloned into VR-1632 using unique *Sfi* I restriction sites, by a strategy similar to that outlined for VR1623 in Example 1 above. The murine kappa light and gamma 1 heavy chain constant regions were amplified from peripheral blood lymphocyte cDNA prepared as above and cloned into VR-1031 to create VR1632. The PCR primers used to amplify the murine kappa light chain constant region were: 5'-GGC GGC GGC CGA TGC GGC CCC AAC TGT ATC CAT CTT CCC A–3' (SEQ ID NO:40) and 5'- GCG GCG ACG CGT TCA ACA CTC ATT CCT GTT GAA G –3'(SEQ ID NO:41). This fragment was cloned as an *Sfi* I – *Mlu* I fragment into VR-1031. The murine gamma 1 constant region was amplified with the PCR primers: 5'- GCG GCC GGC CAA AAC GGC CGC CCC CAT CGG TCT ATC CAC T –3' (SEQ ID NO:42) and 5'- GCG GCG AGA TCT GGA TCT TCA TTT ACC CGG AGT CCG GGA –3' (SEQ ID NO:43) and cloned as an *Sfi* I – *Bgl* II fragment into VR-1031 to form

VR-1632 (SEQ ID NO:4). The PCR cycling conditions for both fragments were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

The light and heavy chain variable regions from the B cell lymphoma cell line RAMOS were amplified and cloned into VR-1632 to serve as a test vaccine construct (Klein G, *et al. Intervirology*, 5:319-334.) This vaccine plasmid is called VR-1642. The RAMOS lambda variable region was PCR amplified with the primers: 5'- GCG GCG GGC CTT GCT GGC CAT GGC CTG GGC TCT GCT G –3' (SEQ ID NO:44) and 5'- GCG GCG GGC CGC ATC GGC CCC TAG GAC GGT CAG CTT GGT G –3' (SEQ ID NO:45) and cloned into VR-1632 as an *Sfi* I fragment. The RAMOS gamma 1 heavy chain constant region was PCR amplified with the primers: 5'- GCG GCG GGC CGC AGC GGC CAA ACA CCT GTG GTT CTT CCT C –3' (SEQ ID NO:46) and 5'- GCG GCG GGC CGT TTT GGC CGA GGA GAC CGT GAC CGT G –3' (SEQ ID NO:47) and cloned into VR-1632 as an *Sfi* I fragment to yield VR-1642 (SEQ ID NO:3). The PCR cycling conditions for both fragments were 94°C for 5 minutes followed by 30 cycles of: 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute.

Measurement of humoral responses in vaccinated individuals.

Tumor Ig protein or isotype matched Igs are captured onto microtiter plates coated with goat anti-human heavy chain antibodies (Biosource International Inc., Camarillo, CA). When the tumor immunoglobulin isotype is an IgG, $F(ab')_2$ fragments are produced by digestion with immobilized pepsin (Pierce, Rockford, IL) and used to coat microtiter plates directly. Preimmunization and postimmunization patient serum are serially diluted and allowed to bind to the target proteins. The binding of anti-Id antibodies is detected by polyclonal goat antihuman IgG antibodies (Biosource International) coupled to horseradish peroxidase (HRP). A response is interpreted as positive when a fourfold increase in anti-Id antibody titer is found when compared to the

preimmunization serum, and to the binding of irrelevant isotype-matched proteins used as specificity targets.

Measurement of T-cell proliferation in response to vaccination by cellular proliferation assay.

Peripheral blood mononuclear cells (PBMC) are obtained by Ficoll Hypaque (Pharmacia, Uppsala, Sweden) density gradient separation of 40 to 60 mL of heparinized blood samples. PBMC are cultured in quadruplicate in media containing either tumor Ig protein or isotype matched, irrelevant protein at concentrations of 0 to 100 μg/mL as previously described. Determination of [^{3}H]-thymidine incorporation is performed over days 5 to 6. A response is interpreted as positive when incorporation of more than two times background is found on two or more occasions.

Statistical analysis.

Freedom from disease progression and survival data are analyzed using Kaplan-Meier analysis and two-sample log rank tests of significance. Freedom from disease progression is measured from the date of last chemotherapy before vaccine treatment to the date of death or last follow-up. The date of last chemotherapy is the reference time point used for these calculations because the impact of vaccine treatments can only be tested when compared to the time of progression from the last proven to cause tumor responses. It is at this point in time in which the relevant baseline clinical status is represented.

Example 3

Prophylactic Application of Flt-3ligand-encoding pDNA in Combination with a Malarial Antigen-encoding pDNA in Humans

Immunogenic compositions comprising (1) pDNA encoding human Flt-3 ligand; and (2) pDNA encoding *Plasmodium falciparum* circumsporozoite protein (PfCSP) are prepared according to the method described above. Human subjects

receive three injections of 0.1, 0.5, 1.0, 2.5, or 5.0 mg of each pDNA in a pharmaceutically acceptable vehicle at 4-week intervals in alternate deltoids. Serum is removed from the subjects and the *Plasmodium falciparum* antibody levels are determined by serial dilution using a standard ELISA assay, as described above. Immune responses of human subjects to the antibody are induced, as indicated by normalized GMT values.

Example 4

Prophylactic Application of Flt-3 ligand-encoding pDNA in Combination with an Influenza A virus Antigen-encoding pDNA in Humans

To prevent influenza virus infection in humans, immunogenic compositions comprising pDNA encoding an influenza A virus hemagglutinin (HA) and pDNA encoding human Flt-3 ligand are prepared according to the method described above. Subjects are injected three times with 0.1, 0.5, 1.0, 2.5, or 5.0 mg of each pDNA in a pharmaceutically acceptable vehicle at 4-week intervals in alternate deltoids. Serum is removed from the humans and antibody levels to the hepatitis antigen are determined by serial dilution using a standard ELISA assay, as described above. Immune responses of the human subjects to the HA antigen are induced, as indicated by normalized GMT values.

Example 5

Treatment Regimen with Flt-3 ligand-encoding pDNA in Combination with Hepatitis Antigen-encoding pDNA for Patients with Chronic Hepatitis B or C

To decrease the chronic infection of Hepatitis B and C in human patients, 0.5-10 mg, preferably 1-5 mg of a pDNA encoding the secreted form of human Flt-3 ligand and 1-30 mg, preferably 10-30 mg pDNA encoding either a hepatitis B virus (HBV) antigen, for example a hepatitis B core antigen (HbcAg), a hepatitis B surface antigen (HBsAg); or a hepatitis C virus (HCV) antigen, for example a HCV nonstructural (NS) protein antigen, a HCV nucleocapsid antigen,

or a HCV envelope glycoprotein antigen, in a pharmacologically acceptable carrier is injected to the patients biweekly or monthly. The therapy regimen is continued for a minimum of 24 weeks during which time the patients are monitored for, for example, levels of serum alanine aminotransferase and clearance of hepatitis C virus (HCV) RNA in HCV patients, and serum HBsAg and HBV DNA are in HBV. Liver biopsies are performed at the end of the treatment period. A successful outcome of the therapy in a HBV or HCV patient is indicated, for example, by a normalization of serum alanine aminotransferase levels, a decrease in serum levels of HBsAg, a disappearance or decrease in detectable virus in the patient's serum, and histological improvement in the liver. In some cases, this therapy is used in conjunction with anti-virals such as lamivudine for HBV and ribavirin for HCV.

Example 6

Treatment Regimen with FLT-3 ligand-encoding pDNA in Combination with IFNω Plasmid DNA for Patients with Chronic Hepatitis B or C

To decrease the chronic infection of Hepatitis B and C in human patients, 0.5-10 mg, preferably 1-5 mg of a pDNA encoding the secreted form of human FLT-3 ligand and 1-50 mg, preferably 10-30 mg of a pDNA encoding IFNω in a pharmacologically acceptable carrier is delivered by injection to patients biweekly or monthly. The therapy regimen is continued for a minimum of 24 weeks during which time the patients are monitored for, for example, levels of serum alanine aminotransferase and clearance of hepatitis C virus (HCV) RNA in HCV patients, and serum HBsAg and HBV DNA for HBV patients. In addition, liver biopsies are performed at the end of the treatment period. A successful outcome of the therapy in HBV or HCV patient is indicated, for example, by a normalization of serum alanine aminotransferase levels, a decrease in serum levels of HbsAg, a disappearance or decrease in detectable virus in the patient's serum, and histological improvement in the liver. In some cases, this

therapy is used in conjunction with anti-virals such as lamivudine for HBV and ribavirin for HCV.

Example 7

Preparation of Cytofectin Formulations

(±)-N-(3-aminopropyl)-N,N-dimethyl-2,3-bis(*syn*-9-tetradeceneyloxy)-1-propanaminium bromide (GAP-DMORIE) was synthesized using the published procedure for preparing the analogue cytofectin GAP-DLRIE (Wheeler *et al., Proc. Natl. Acad. Sci.* 93, 11454-11459 (1996)). Specifically, substituting *syn*-9-tetradecenyl methane sulfonate for dodecenyl methane sulfonate in the initial *bis*-alkylation of 3-dimethylamino-1,2-propanediol yielded the desired dialkenyl amine. Quatranization with 3-bromopropylphthalimide, followed by deprotection of the protected primary amine with hydrazine and extractive purification and sub-micron filtration afforded pure GAP-DMORIE as judged by analytical thin layer chromatography. Product identity was confirmed using high resolution proton NMR and infrared (IR) spectroscopies.

Both the 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-diphytanoyl-*sn*-glycero-3-phosphoethanolamine (DPyPE) were purchased as chloroform solutions from Avanti Polar Lipids (Alabaster, Alabama). Cytofectin:co-lipid liposomes were prepared using the rehydrated thin-film method described above. Cytofectin formulation/pDNA complexes were prepared by mixing equivalent volumes of a pDNA solution and a solution of cytofectin/co-lipid liposomes.

What Is Claimed Is:

1. A pharmaceutical composition comprising:

(a) about 1 ng to 10 mg of a nucleic acid molecule comprising a first polynucleotide which hybridizes, at 42°C in 50% formamide, 5x SSC, 50 mM sodium phosphate, 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing at 65°C in 0.1x SSC and 0.1% SDS (w/v), to a reference nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and the complement of any of said sequences, wherein said first polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate;

(b) about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding one or more antigens or one or more cytokines; and

(c) a pharmaceutically acceptable carrier,

wherein said first and second polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof.

2. The pharmaceutical composition of claim 1, wherein said first polynucleotide encodes a polypeptide comprising 15 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24.

3. The pharmaceutical composition of claim 2, wherein said first polynucleotide encodes a polypeptide comprising at least 30 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID

NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24.

4. The pharmaceutical composition of claim 3, wherein said first polynucleotide encodes a polypeptide comprising at least 50 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24.

5. The pharmaceutical composition of claim 4, wherein said first polynucleotide encodes a polypeptide comprising at least 150 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24.

6. The pharmaceutical composition of Claim 1, wherein said first polynucleotide encodes amino acids 28 to 163 of SEQ ID NO:8.

7. The pharmaceutical composition of Claim 6, wherein said first polynucleotide encodes amino acids 1 to 163 of SEQ ID NO:8.

8. The pharmaceutical composition of Claim 6, wherein said first polynucleotide encodes amino acids 28 to 189 of SEQ ID NO:8.

9. The pharmaceutical composition of Claim 8, wherein said first polynucleotide encodes amino acids 1 to 189 of SEQ ID NO:8.

10. The pharmaceutical composition of Claim 8, wherein said first polynucleotide encodes amino acid residues selected from the group consisting

of amino acids 28 to 231 of SEQ ID NO:8, and amino acids 28 to 232 of SEQ ID NO:13.

11. The pharmaceutical composition of Claim 9, wherein said first polynucleotide encodes amino acid residues selected from the group consisting of amino acids 1 to 231 of SEQ ID NO:8, and amino acids 1 to 232 of SEQ ID NO:13.

12. The pharmaceutical composition of Claim 6, wherein said first polynucleotide encodes amino acids 28 to 220 of SEQ ID NO:11.

13. The pharmaceutical composition of Claim 12, wherein said first polynucleotide encodes amino acids 1 to 220 of SEQ ID NO:11.

14. The pharmaceutical composition of Claim 6, wherein said first polynucleotide encodes amino acids 28 to 172 of SEQ ID NO:17.

15. The pharmaceutical composition of Claim 14, wherein said first polynucleotide encodes amino acids 1 to 172 of SEQ ID NO:17.

16. The pharmaceutical composition of Claim 1, wherein said first polynucleotide encodes amino acids 27 to 160 of SEQ ID NO:19.

17. The pharmaceutical composition of Claim 16, wherein said first polynucleotide encodes amino acids 1 to 160 of SEQ ID NO:19.

18. The pharmaceutical composition of Claim 16, wherein said first polynucleotide encodes amino acids 27 to 185 of SEQ ID NO:19.

19. The pharmaceutical composition of Claim 18, wherein said first polynucleotide encodes amino acids 1 to 185 of SEQ ID NO:19.

20. The pharmaceutical composition of Claim 18, wherein said first polynucleotide encodes amino acids 27 to 235 of SEQ ID NO:19.

21. The pharmaceutical composition of Claim 19, wherein said first polynucleotide encodes amino acids 1 to 235 of SEQ ID NO:19.

22. The pharmaceutical composition of Claim 16, wherein said first polynucleotide encodes amino acids 27 to 178 of SEQ ID NO:21.

23. The pharmaceutical composition of Claim 22, wherein said first polynucleotide encodes amino acids 1 to 178 of SEQ ID NO:21.

24. The pharmaceutical composition of Claim 1, wherein said first polynucleotide encodes amino acids 27 to 185 of SEQ ID NO:23.

25. The pharmaceutical composition of Claim 24, wherein said first polynucleotide encodes amino acids 1 to 185 of SEQ ID NO:23.

26. The pharmaceutical composition of Claim 24, wherein said first polynucleotide encodes amino acids 27 to 235 of SEQ ID NO:23.

27. The pharmaceutical composition of Claim 26, wherein said first polynucleotide encodes amino acids 1 to 235 of SEQ ID NO:23.

28. The pharmaceutical composition of claim 1, wherein said first polynucleotide encodes three amino acid regions comprising:

(a) amino acid residues 34 to 41 of SEQ ID NO:19 arranged consecutively,

(b) amino acid residues 107 to 113 of SEQ ID NO:19 arranged consecutively, and

(c) amino acid residues 142 to 150 of SEQ ID NO:19 arranged consecutively.

29. The pharmaceutical composition of claim 1, wherein said first polynucleotide encodes a polypeptide selected from the group consisting of:

(a) a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:19, is identical to amino acids 27 to 160 of SEQ ID NO:19, and

(b) a polypeptide which, except for at least one amino acid substitution at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147 of SEQ ID NO:23, is identical to amino acids 27 to 185 of SEQ ID NO:23;

wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide.

30. A pharmaceutical composition comprising:

(a) about 1 ng to 10 mg of a nucleic acid molecule comprising a first polynucleotide wherein said first polynucleotide encodes a first polypeptide which, except for at least one but not more than 20 amino acid substitutions, deletions, or insertions, is identical to a second polypeptide selected from the group consisting of amino acids 28 to 163 of SEQ ID NO:8, amino acids 27 to 160 of SEQ ID NO:19, and amino acids 27 to 185 of SEQ ID NO:23; wherein said first polypeptide has immunity-enhancing activity when administered to a vertebrate;

(b) about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding one or more antigens or one or more cytokines; and

(c) a pharmaceutically acceptable carrier,

wherein said first and second polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof.

31. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 1 to 163 of SEQ ID NO:8.

32. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 28 to 189 of SEQ ID NO:8.

33. The pharmaceutical composition of Claim 32, wherein said second polypeptide comprises amino acids 1 to 189 of SEQ ID NO:8.

34. The pharmaceutical composition of Claim 32, wherein said second polypeptide is selected from the group consisting of amino acids 28 to 231 of SEQ ID NO:8, and amino acids 28 to 232 of SEQ ID NO:13.

35. The pharmaceutical composition of Claim 34, wherein said second polypeptide is selected from the group consisting of amino acids 1 to 231 of SEQ ID NO:8, and amino acids 1 to 232 of SEQ ID NO:13.

36. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 28 to 220 of SEQ ID NO:11.

37. The pharmaceutical composition of Claim 36, wherein said second polypeptide comprises amino acids 1 to 220 of SEQ ID NO:11.

38. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 28 to 172 of SEQ ID NO:17.

39. The pharmaceutical composition of Claim 38, wherein said second polypeptide comprises amino acids 1 to 172 of SEQ ID NO:17.

40. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 1 to 160 of SEQ ID NO:19.

41. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 27 to 185 of SEQ ID NO:19.

42. The pharmaceutical composition of Claim 41, wherein said second polypeptide comprises amino acids 1 to 185 of SEQ ID NO:19.

43. The pharmaceutical composition of Claim 41, wherein said second polypeptide comprises amino acids 27 to 235 of SEQ ID NO:19.

44. The pharmaceutical composition of Claim 42, wherein said second polypeptide comprises amino acids 1 to 235 of SEQ ID NO:19.

45. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 27 to 178 of SEQ ID NO:21.

46. The pharmaceutical composition of Claim 45, wherein said second polypeptide comprises amino acids 1 to 178 of SEQ ID NO:21.

47. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 1 to 185 of SEQ ID NO:23.

48. The pharmaceutical composition of Claim 30, wherein said second polypeptide comprises amino acids 27 to 235 of SEQ ID NO:23.

49. The pharmaceutical composition of Claim 48, wherein said second polypeptide comprises amino acids 1 to 235 of SEQ ID NO:23.

50. The pharmaceutical composition of Claim 30, wherein the number of amino acid substitutions, deletions, or insertions is not more than 10.

51. The pharmaceutical composition of Claim 30, wherein the number of amino acid substitutions, deletions, or insertions is not more than 5.

52. The pharmaceutical composition of Claim 30, wherein the number of amino acid substitutions, deletions, or insertions is not more than 1.

53. The pharmaceutical composition of claim 30, wherein said amino acid substitutions, deletions or insertions do not occur in regions identical to amino acids 34 to 41, 107 to 113, and 142 to 150 of SEQ ID NO:19.

54. The pharmaceutical composition of claim 30, wherein said first polynucleotide encodes a polypeptide selected from the group consisting of:

(a) a polypeptide having amino acids 27 to 160 of SEQ ID NO:19, wherein at least one amino acid substitution occurs at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147, and

(b) a polypeptide having amino acids 27 to 185 of SEQ ID NO:23, wherein at least one amino acid substitution occurs at an amino acid position selected from the group consisting of amino acid residues 34, 110, 144, and 147;

wherein said amino acid substitution increases the immunity enhancing activity of said polypeptide.

55. A pharmaceutical composition comprising:

(a) about 1 ng to 10 mg of a nucleic acid molecule comprising a first polynucleotide, wherein said first polynucleotide encodes an amino acid sequence that is at least 90% identical to a reference amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, and SEQ ID NO:24, wherein % identity is determined using the Bestfit program with default parameters, and wherein said first polynucleotide encodes a polypeptide having immunity-enhancing activity when administered to a vertebrate;

(b) about 1 ng to 30 mg of a nucleic acid molecule comprising a second polynucleotide encoding one or more antigens or one or more cytokines; and

(c) a pharmaceutically acceptable carrier,

wherein said first and second polynucleotides are non-infectious and non-integrating, and are operably associated with control sequences which direct the expression thereof.

56. The pharmaceutical composition of claim 55, wherein said first polynucleotide encodes an amino acid sequence that is at least 95% identical to said reference amino acid sequence.

57. The pharmaceutical composition of claim 55, wherein said first polynucleotide encodes an amino acid sequence that is at least 97% identical to said reference amino acid sequence.

58. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein the nucleic acid molecule of (a) is selected from the group consisting of VR6200 (SEQ ID NO:6) and VR6230 (SEQ ID NO:39).

59. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said antigen is selected from the group consisting of a viral antigen, a bacterial antigen, a protozoan parasite antigen, a helminth parasite antigen, a fungal antigen, an ectoparasite antigen, a tumor associated antigen, and a self antigen associated with autoimmunity.

60. The pharmaceutical composition of claim 59, wherein said viral antigen is derived from a virus selected from the group consisting of adenoviruses, alphaviruses, apthoviruses, caliciviruses, coronaviruses, coxsackieviruses, distemper viruses, Ebola viruses, enteroviruses, flaviviruses, hepatitis viruses, herpesviruses, immunodeficiency viruses, infectious peritonitis viruses, influenza viruses, leukemia viruses, Marburg viruses, oncogenic viruses, orthomyxoviruses, papilloma viruses, parainfluenza viruses, paramyxoviruses, parvoviruses, pestiviruses, picorna viruses, pox viruses, rabies viruses, reoviruses, respiratory syncytial viruses, retroviruses, rhinoviruses, rotaviruses, cancer-causing viruses or cancer-related viruses.

61. The pharmaceutical composition of Claim 59, wherein said bacterial antigen is derived from an organism having a genus selected from the group consisting of *Actinomyces, Bacillus, Bacteroides, Bordetella, Bartonella, Borrelia, Brucella, Campylobacter, Capnocytophaga, Chlamydia, Clostridium, Corynebacterium, Coxiella, Dermatophilus, Enterococcus, Ehrlichia, Escherichia, Francisella, Fusobacterium, Haemobartonella, Haemophilus, Helicobacter, Klebsiella*, L-form bacteria, *Leptospira, Listeria, Mycobacteria, Mycoplasma, Neisseria, Neorickettsia, Nocardia, Pasteurella, Peptococcus, Peptostreptococcus, Pneumococcus, Proteus, Pseudomonas, Rickettsia,*

Rochalimaea, Salmonella, Shigella, Staphylococcus, Streptococcus, Treponema, and *Yersinia.*

62. The pharmaceutical composition of claim 59, wherein said protozoan parasite antigen is derived from an organism having a genus selected from the group consisting of *Babesia*, *Balantidium*, *Besnoitia*, *Cryptosporidium*, *Eimeria*, *Encephalitozoon*, *Entamoeba*, *Giardia*, *Hammondia, Hepatozoon*, *Isospora*, *Leishmania*, *Microsporidia*, *Neospora*, *Nosema*, *Pentatrichomonas*, *Plasmodium*, *Pneumocystis*, *Sarcocystis*, *Schistosoma*, *Theileria*, *Toxoplasma*, and *Trypanosoma*.

63. The pharmaceutical composition of claim 59, wherein said helminth parasite antigen is derived from an organism having a genus selected from the group consisting of *Acanthocheilonema*, *Aelurostrongylus*, *Ancylostoma*, *Angiostrongylus*, *Ascaris*, *Brugia*, *Bunostomum*, *Capillaria*, *Chabertia*, *Cooperia*, *Crenosoma*, *Dictyocaulus*, *Dioctophyme*, *Dipetalonema*, *Diphyllobothrium*, *Diplydium*, *Dirofilaria*, *Dracunculus*, *Enterobius*, *Filaroides*, *Haemonchus*, *Lagochilascaris*, *Loa*, *Mansonella*, *Muellerius*, *Nanophyetus*, *Necator*, *Nematodirus*, *Oesophagostomum*, *Onchocerca*, *Opisthorchis*, *Ostertagia*, *Parafilaria*, *Paragonimus*, *Parascaris*, *Physaloptera*, *Protostrongylus*, *Setaria*, *Spirocerca*, *Spirometra*, *Stephanofilaria*, *Strongyloides*, *Strongylus*, *Thelazia*, *Toxascaris, Toxocara*, *Trichinella*, *Trichostrongylus*, *Trichuris*, *Uncinaria*, and *Wuchereria*.

64. The pharmaceutical composition of claim 59, wherein said fungal antigen is derived from an organism having a genus selected from the group consisting of *Absidia, Acremonium, Alternaria, Aspergillus, Basidiobolus, Bipolaris, Blastomyces, Candida, Coccidioides, Conidiobolus, Cryptococcus, Curvalaria, Epidermophyton, Exophiala, Geotrichum, Histoplasma, Madurella, Malassezia, Microsporum, Moniliella, Mortierella, Mucor, Paecilomyces,*

Penicillium, Phialemonium, Phialophora, Prototheca, Pseudallescheria, Pseudomicrodochium, Pythium, Rhinosporidium, Rhizopus, Scolecobasidium, Sporothrix, Stemphylium, Trichophyton, Trichosporon, and *Xylohypha*.

65. The pharmaceutical composition of claim 59, wherein said ectoparasite antigen is derived from an organism selected from the group consisting of fleas, ticks, mosquitos, sand flies, black flies, horse flies, horn flies, deer flies, tsetse flies, stable flies, myiasis-causing flies, biting gnats, ants, spiders, lice, mites, bed bugs, and kissing bugs.

66. The pharmaceutical composition of claim 59, wherein said tumor-associated antigen comprises a tumor-specific immunoglobulin variable region, a GM2 antigen, a Tn antigen, an sTn antigen, a Thompson-Friedenreich antigen (TF), a Globo H antigen, an Le(y) antigen, a MUC1 antigen, a MUC2 antigen, a MUC3 antigen, a MUC4 antigen, a MUC5AC antigen, a MUC5B antigen, a MUC7 antigen, a carcinoembryonic antigen, a beta chain of human chorionic gonadotropin (hCG beta) antigen, a HER2/neu antigen, a PSMA antigen, a EGFRvIII antigen, a KSA antigen, a PSA antigen, a PSCA antigen, a GP100 antigen, a MAGE 1 antigen, a MAGE 2 antigen, a TRP 1 antigen, a TRP 2 antigen, and a tyrosinase antigen.

67. The pharmaceutical composition of claim 66, wherein said tumor-associated antigen comprises a B-cell lymphoma-specific idiotype determinant.

68. The pharmaceutical composition of claim 67, wherein said tumor specific antigen further comprises an immunoglobulin constant region.

69. The pharmaceutical composition of claim 67, wherein said second polynucleotide encoding said tumor-associated antigen is polycistronic.

70. The pharmaceutical composition of claim 69, wherein said second polynucleotide comprises

(a) a first cistron encoding a protein comprising a light chain variable region of a B-cell lymphoma immunoglobulin having a tumor-specific idiotype determinant, fused to a constant region, and

(b) a second cistron encoding a protein comprising a heavy chain variable region of a B-cell lymphoma immunoglobulin having a tumor-specific idiotype determinant, fused to a constant region.

71. The pharamaceutical composition of claim 70, wherein said constant region is derived from a heterologous species relative to said variable region.

72. The pharmaceutical composition of claim 69 wherein said second polynucleotide comprises two cistrons organized in a transcription unit under the control of a single promoter, and wherein said second polynucleotide further comprises an internal ribosome entry site positioned between said cistrons.

73. The pharmaceutical composition of claim 72, wherein said second polynucleotide is selected from the group consisting of VR1623 (SEQ ID NO:1) and VR1642 (SEQ ID NO:3).

74. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said first and second polynucleotides are present in a single nucleic acid molecule.

75. The pharmaceutical composition of claim 74, wherein said nucleic acid molecule encodes a fusion protein comprising a Flt-3 ligand and one or more antigens or one or more cytokines.

76. The pharmaceutical composition of claim 75, wherein said fusion protein comprises a Flt-3 ligand and a B-cell lymphoma-specific idiotype determinant.

77. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein the nucleic acid molecule of (a) comprises VR6230 (SEQ ID NO:39), and the nucleic acid molecule of (b) comprises VR1642 (SEQ ID NO:3).

78. The pharmaceutical composition of any one of claims 1, 30, or 55, further comprising a cationic lipid.

79. The pharmaceutical composition of claim 78, wherein said cationic lipid comprises a compound selected from the group consisting of DMRIE, GAP-DMORIE and GAP-DLRIE.

80. The pharmaceutical composition of claim 78, wherein said cationic lipid further comprises one or more co-lipids.

81. The pharmaceutical composition of claim 80, wherein said co-lipids are selected from the group consisting of DOPE, DPyPE, and DMPE.

82. The pharmaceutical composition of claim 80, comprising GAP-DMORIE and DPyPE.

83. The pharmaceutical composition of claim 80, wherein the cationic lipid:co-lipid molar ratio ranges from about 2:1 to 1:2.

84. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said control sequences are selected from the group consisting of a

promoter, an enhancer, an operator, a repressor, and a transcription termination signal.

85. The pharmaceutical composition of claim 84, wherein said control sequence is a promoter selected from the group consisting of a cytomegalovirus promoter, a simian virus 40 promoter, and a retrovirus promoter.

86. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said first and second polynucleotides are DNA.

87. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said first and second polynucleotides are RNA.

88. The pharmaceutical composition of any one of claims 1, 30, or 55, wherein said first and second polynucleotides comprise one or more regions regulating gene expression.

89. The pharmaceutical composition of claim 88, wherein said region regulating gene expression is cell specific or tissue specific.

90. The pharmaceutical composition of claim 89, wherein said region is tumor cell or tumor tissue specific.

91. The pharmaceutical composition of any one of claims 1, 30, or 55, further comprising about 1 ng to 10 mg of a nucleic acid molecule comprising a third polynucleotide encoding a cytokine, or active fragment thereof, wherein said third polynucleotide is non-infectious and non-integrating, and is operably associated with control sequences which direct the expression thereof.

92. The pharmaceutical composition of claim 91, wherein said cytokine is selected from the group consisting of GM-CSF, G-CSF, M-CSF, CSF, erythropoietin, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, IFNα, IFNβ, IFNω, IFNτ IFNγ, IFN γ inducing factor I, TGF-β, RANTES, MIP-1 α, MIP-1 β, LEIF, SDF-1, and MCP-3.

93. A method for enhancing an immune response in a vertebrate, said method comprising administering to a tissue of said vertebrate the pharmaceutical composition of any one of claims 1, 30, or 55, wherein said first and second polynucleotides are expressed *in vivo* in an amount effective for a polypeptide expressed by said first polynucleotide to enhance the immunogenicity of said one or more antigens or said one or more cytokines.

94. The method of claim 93, wherein said antigen is selected from the group consisting of a viral antigen, a bacterial antigen, a protozoan parasite antigen, a helminth parasite antigen, a fungal antigen, an ectoparasite antigen, a tumor associated antigen, and a self antigen associated with autoimmunity.

95. The method of claim 93, wherein said tissue is selected from the group consisting of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, tongue and connective tissue.

96. The method of claim 93, wherein said first and second polynucleotides are administered onto a mucosal surface.

97. The method of claim 95, wherein said tissue is muscle.

98. The method of claim 97, wherein said tissue is skeletal muscle, smooth muscle, or myocardium.

99. The method of claim 93, wherein said first and second polynucleotides are administered intravenously.

100. The method of claim 93, wherein said first and second polynucleotides are administered intramuscularly.

101. The method of claim 93, wherein said administration comprises intramuscular injection.

102. The method of claim 93, wherein said first and second polynucleotides are administered by a route selected from the group consisting of transdermal, interdermal, subcutaneous, oral, intraocular, vaginal, rectal, and inhaled.

103. The method of claim 93, wherein said administration is mediated by a catheter.

104. The method of claim 93, wherein said administration is mediated by a device selected from the group consisting of a particle accelerator, a pump, an intradermal applicator, a biolistic injector, a pneumatic injector, a sponge depot, a pill, and a tablet.

105. The method of claim 94, wherein said vertebrate is a mammal.

106. The method of claim 105, wherein said mammal is a human.

107. The method of claim 105, wherein said construct is free from association with transfection-facilitating proteins, viral particles, liposomes, cationic lipids, and calcium phosphate precipitating agents.

108. A method of suppressing tumor growth in a mammal, comprising administering into a tissue of said mammal the pharmaceutical composition of any of claims 1, 30, or 55, wherein the growth of said tumor is suppressed.

109. The method of claim 108, wherein said tumor is selected from the group consisting of melanoma, glioma, and lymphoma.

110. The method of claim 108, wherein said composition is administered intramuscularly to treat B-cell lymphoma.

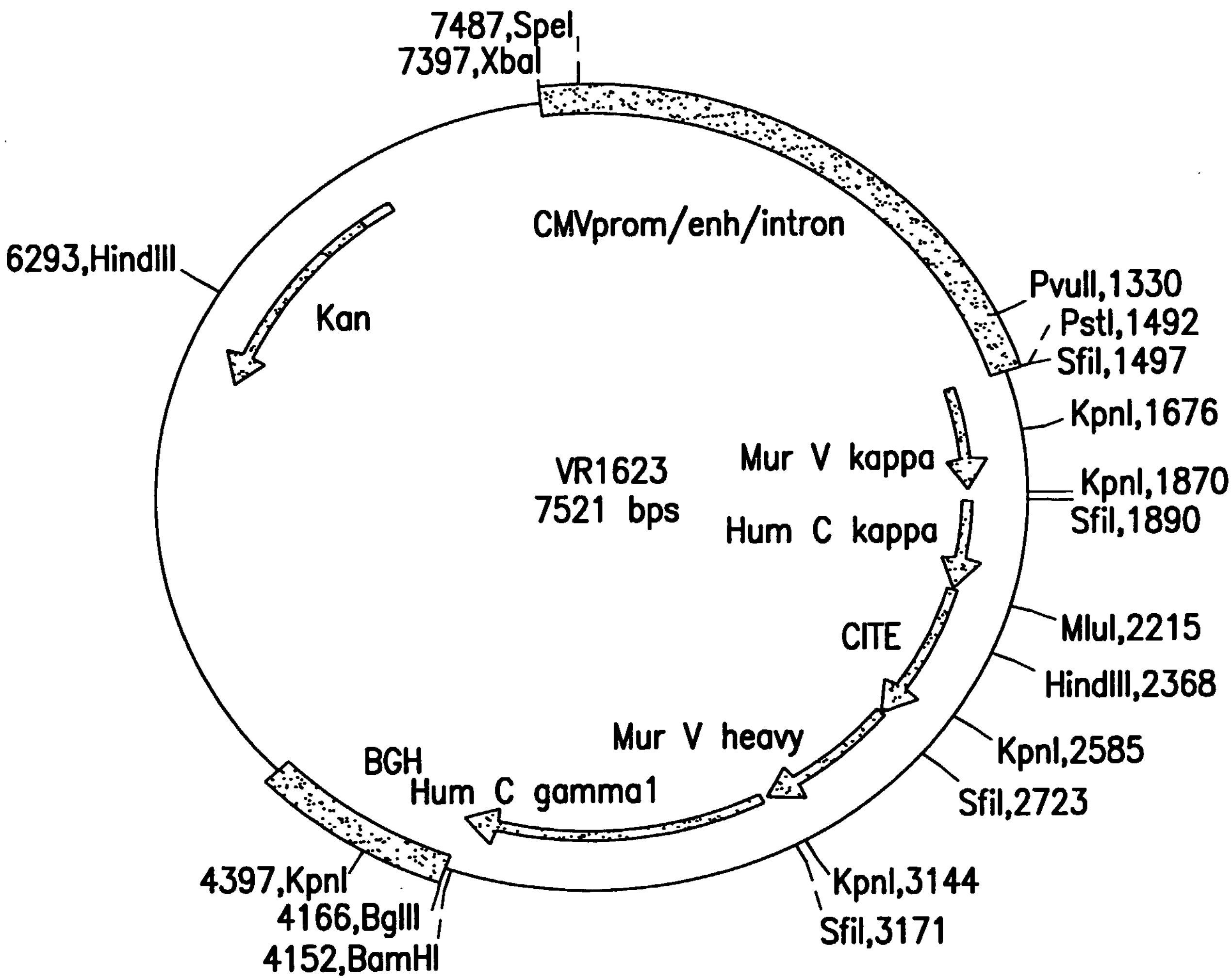
111. A method of suppressing growth of B-cell lymphoma in a mammal comprising administering into a tissue of said mammal the pharmaceutical composition of Claim 70.

112. The method of claim 108, further comprising one or more additional cancer treatment methods selected from the group consisting of surgery, radiation therapy, chemotherapy, immunotherapy, and gene therapy.

113. The method of claim 112, wherein said composition is administered prior to the commencement of said one or more additional cancer treatment methods.

114. The method of claim 112, wherein said composition is administered during the practice of said one or more additional cancer treatment methods.

115. The method of claim 112, wherein said composition is administered after the end of said one or more additional cancer treatment methods.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7397,XbaI
CMVprom/enh/intron
6293,HindIII
Kan
PvuII,1330
PstI,1492
SfiI,1497
KpnI,1676
VR1623
7521 bps
Mur V kappa
KpnI,1870
SfiI,1890
Hum C kappa
MluI,2215
CITE
HindIII,2368
Mur V heavy
KpnI,2585
BGH
Hum C gamma1
SfiI,2723
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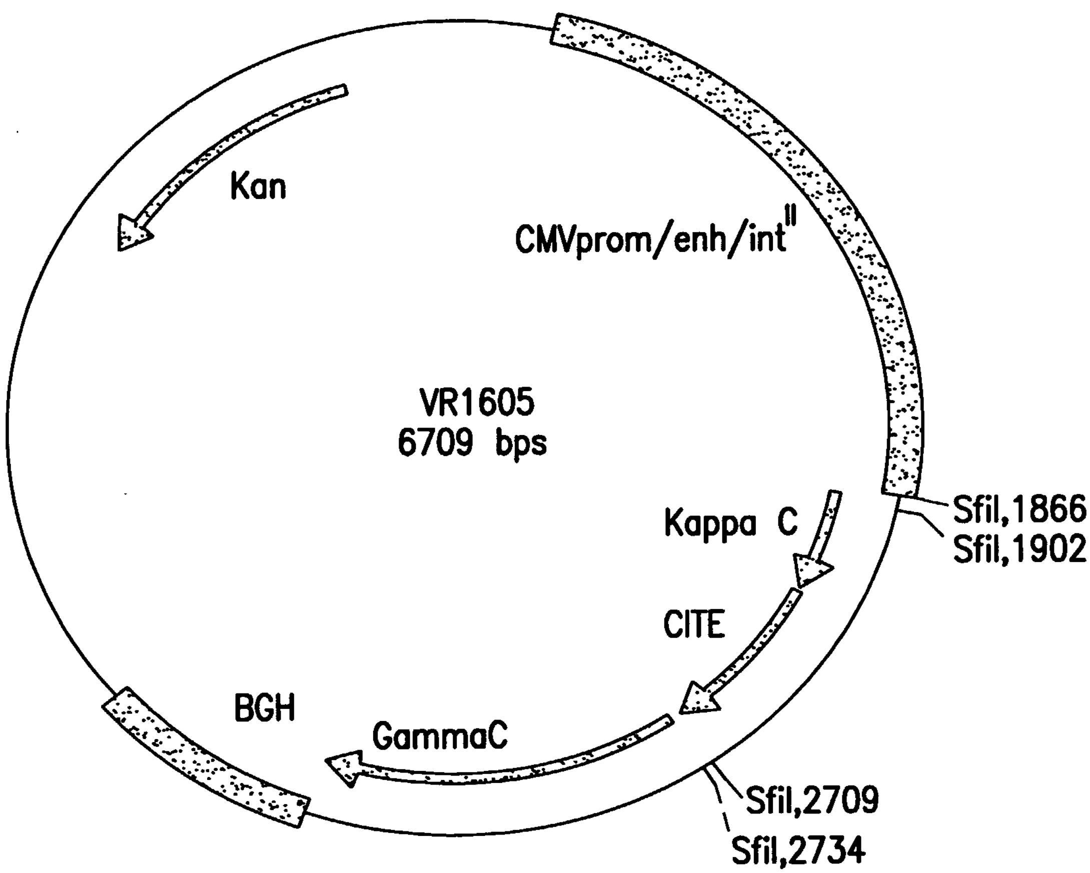
4152,BamHI
KpnI,3144
SfiI,3171

FIG. 1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VR1605
6709 bps
SfiI,1866
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Kappa C
CITE
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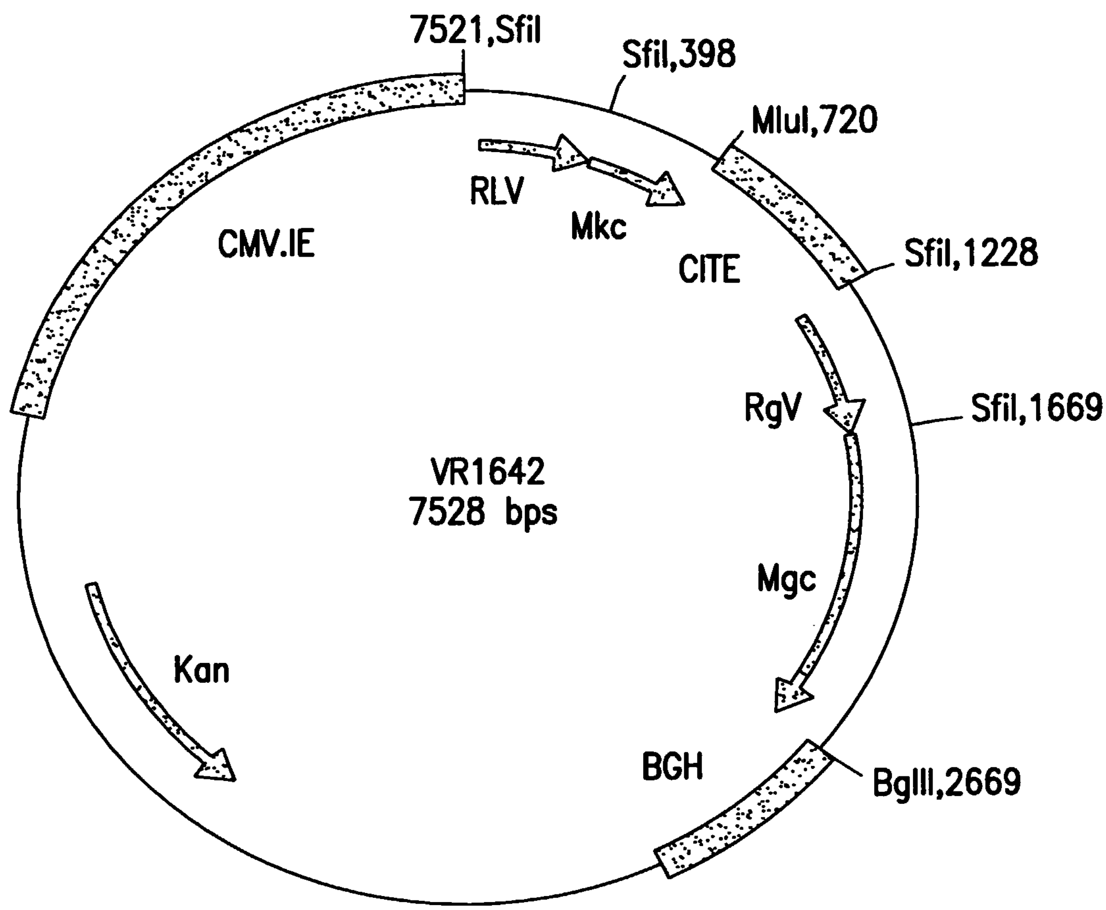
GammaC
SfiI,2709
SfiI,2734

FIG. 2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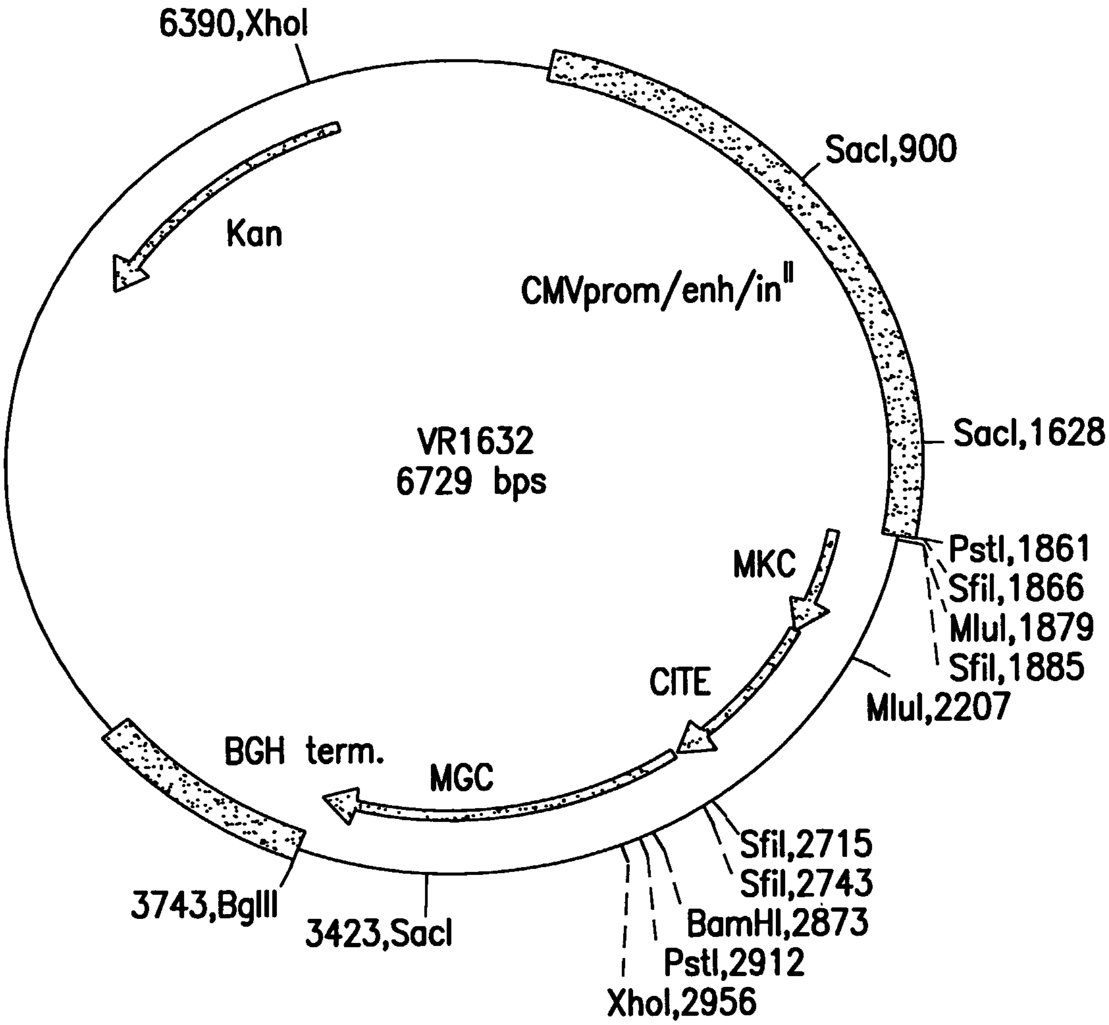
SfiI,398
MluI,720
SfiI,1228
SfiI,1669
BglII,2669
RLV
Mkc
CITE
CMV.IE
RgV
VR1642
7528 bps
Mgc
Kan
BGH

FIG. 3



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SacI,900
Kan
CMVprom/enh/in II
VR1632
6729 bps
SacI,1628
PstI,1861
SfiI,1866
MluI,1879
SfiI,1885
MKC
CITE
MluI,2207
BGH term.
MGC
SfiI,2715
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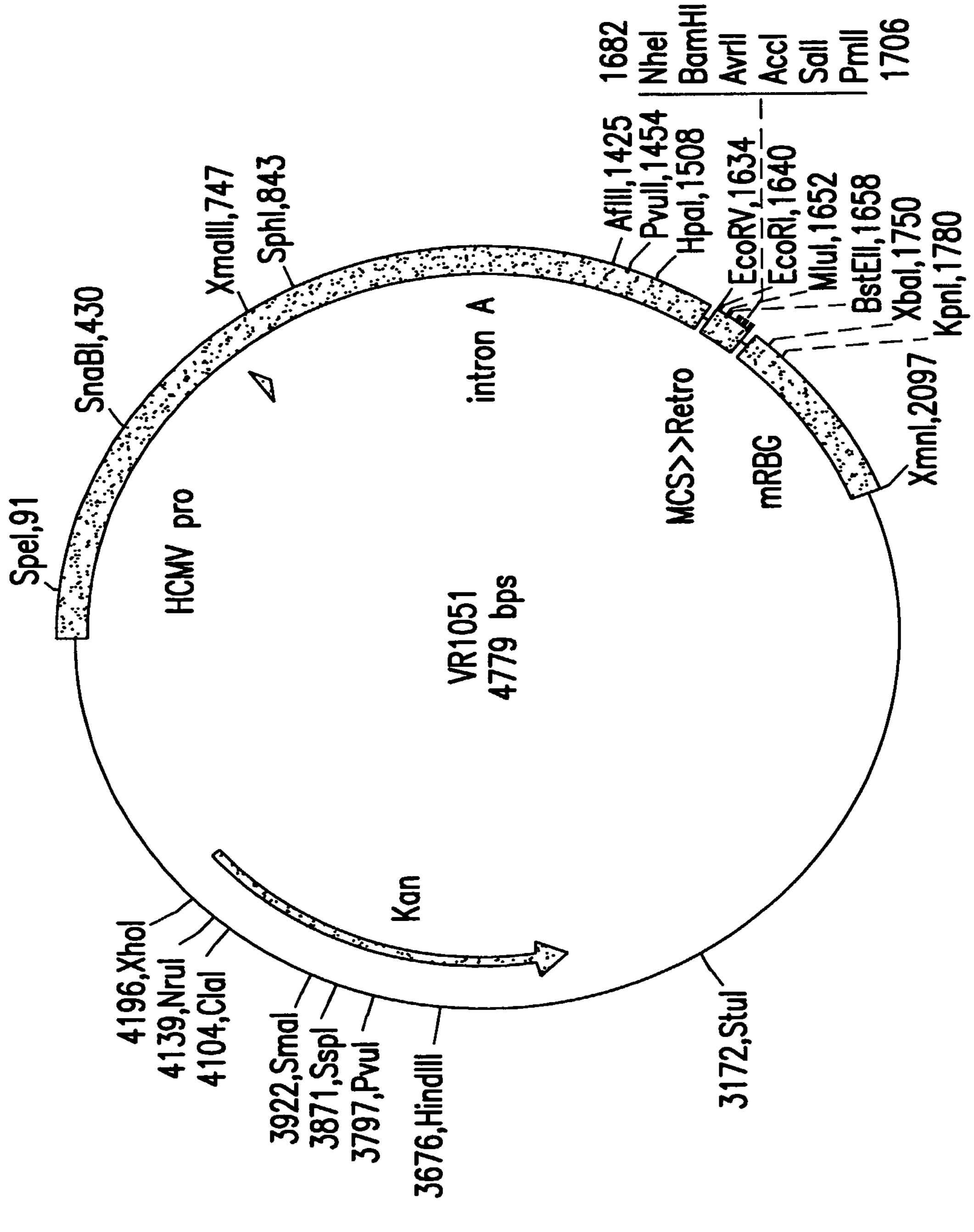
BamHI,2873
PstI,2912
XhoI,2956
3743,BglII
3423,SacI

FIG. 4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HCMV pro
intron A
MCS>>Retro
mRBG
Kan
SpeI,91
SnaBI,430
XmaIII,747
SphI,843
AflII,1425
PvuII,1454
HpaI,1508
EcoRV,1634
EcoRI,1640
MluI,1652
BstEII,1658
XbaI,1750
KpnI,1780
XmnI,2097
1682
NheI
BamHI
AvrII
AccI
SalI
PmlI
1706
3172,StuI
3676,HindIII
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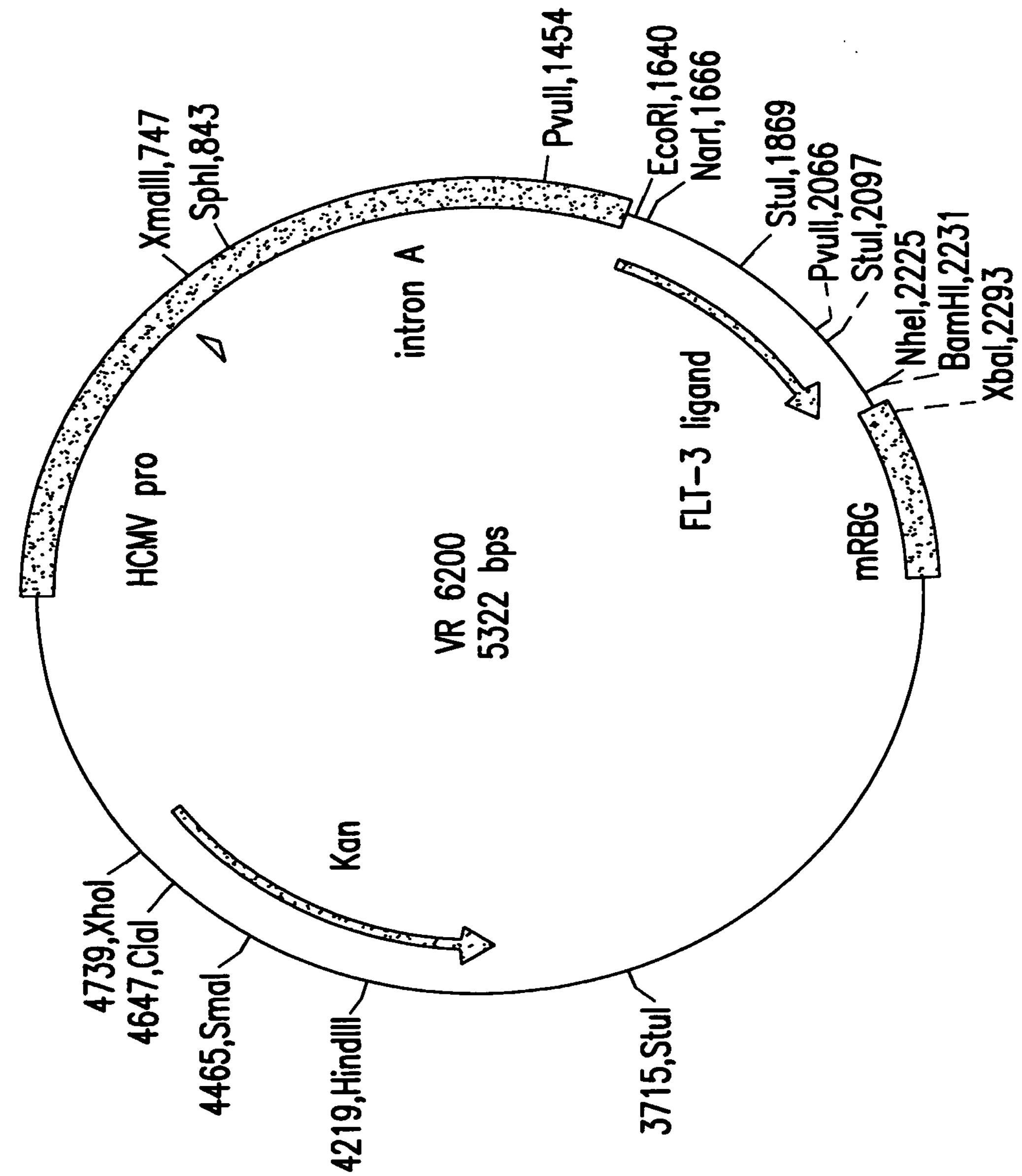
3871,SspI
3922,SmaI
4104,ClaI
4139,NruI
4196,XhoI

FIG. 5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EcoRI,1640
NarI,1666
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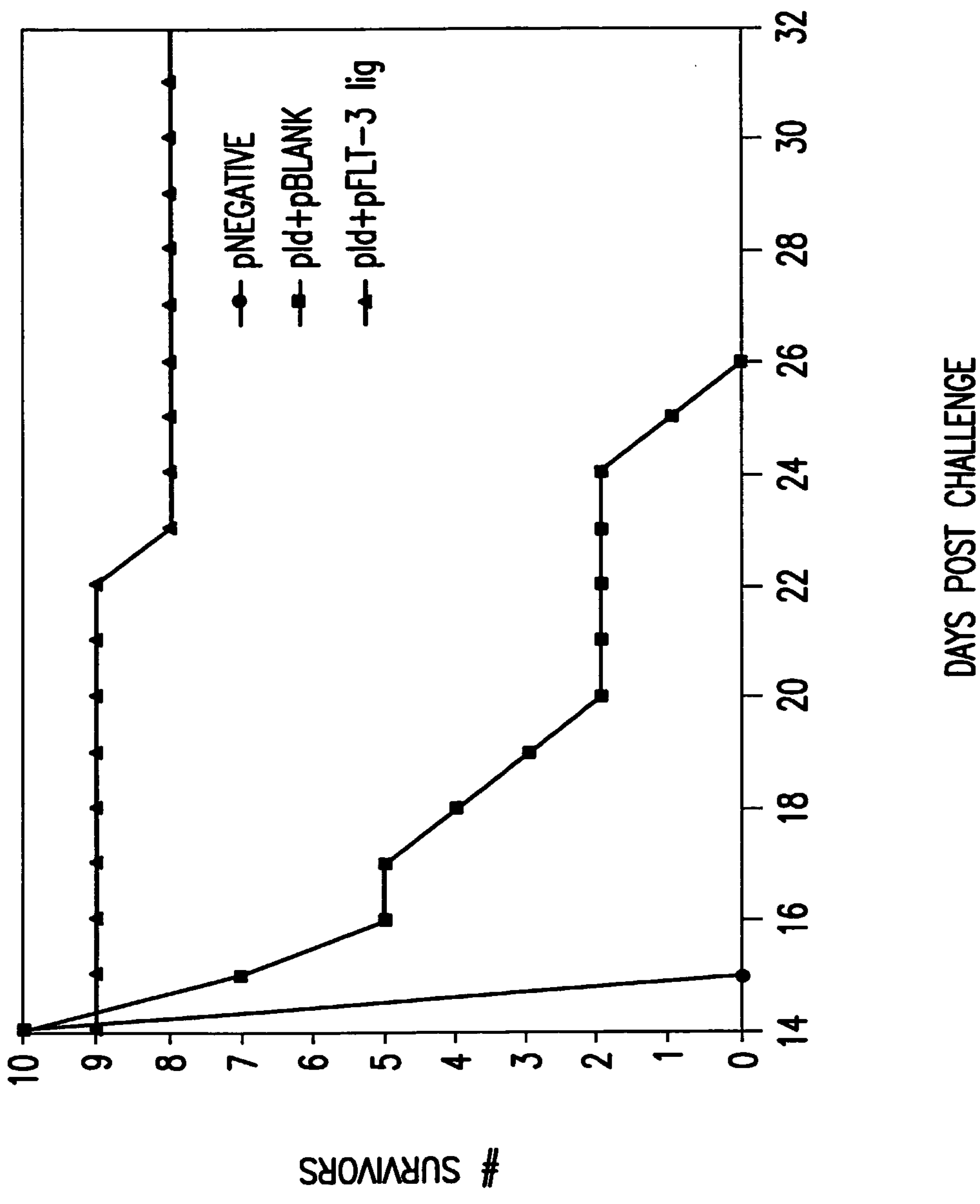
PvuII,2066
StuI,2097
NheI,2225
BamHI,2231
XbaI,2293
intron A
HCMV pro
FLT-3 ligand
mRBG
VR 6200
5322 bps
Kan
4739,XhoI
4647,ClaI
4465,SmaI
4219,HindIII
3715,StuI

FIG. 6



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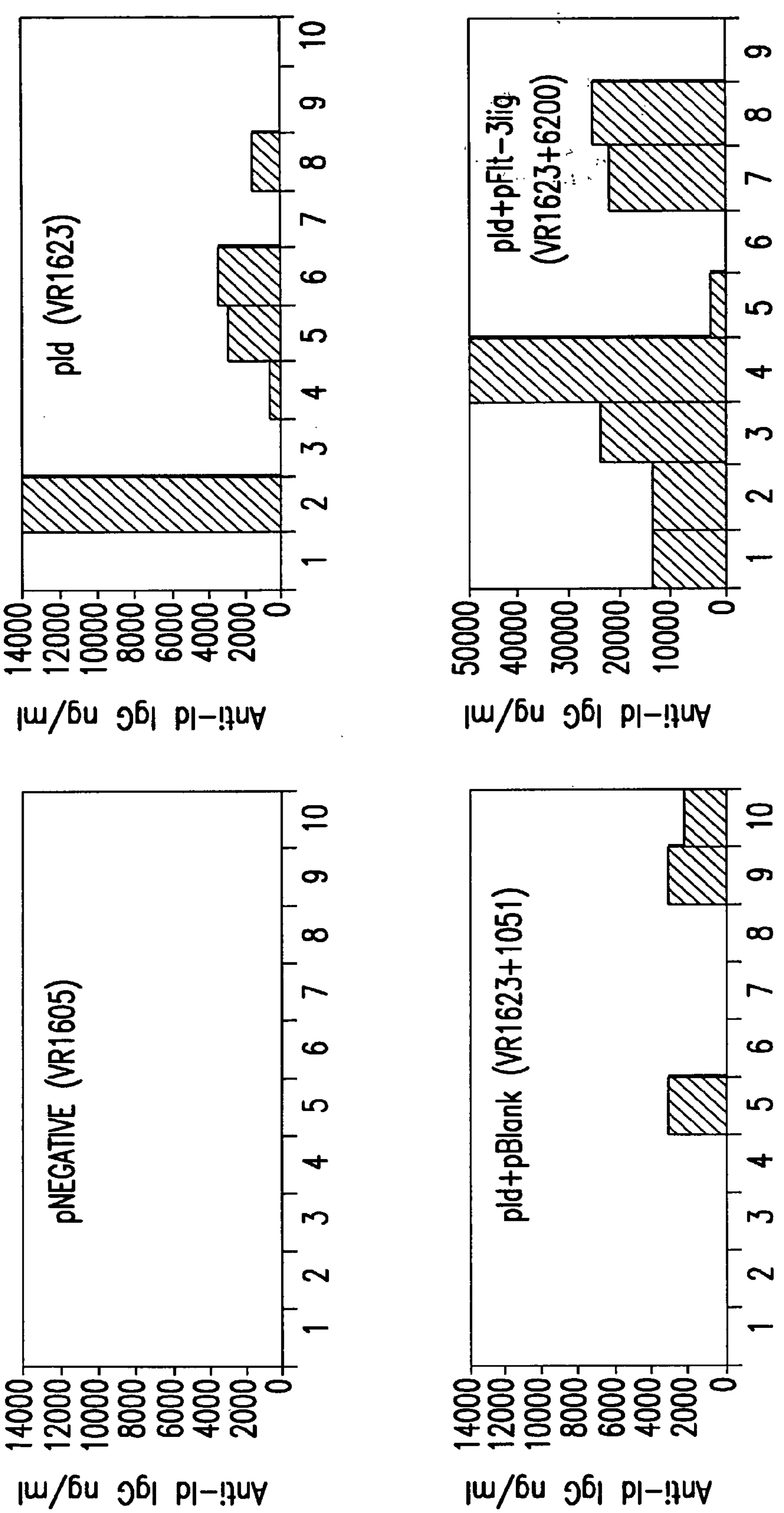
10
9
8
7
6
5
4
3
2
1
0
14
16
18
20
22
24
26
28
30
32
DAYS POST CHALLENGE
pNEGATIVE
pId+pBLANK
pId+pFLT-3 lig

FIG. 7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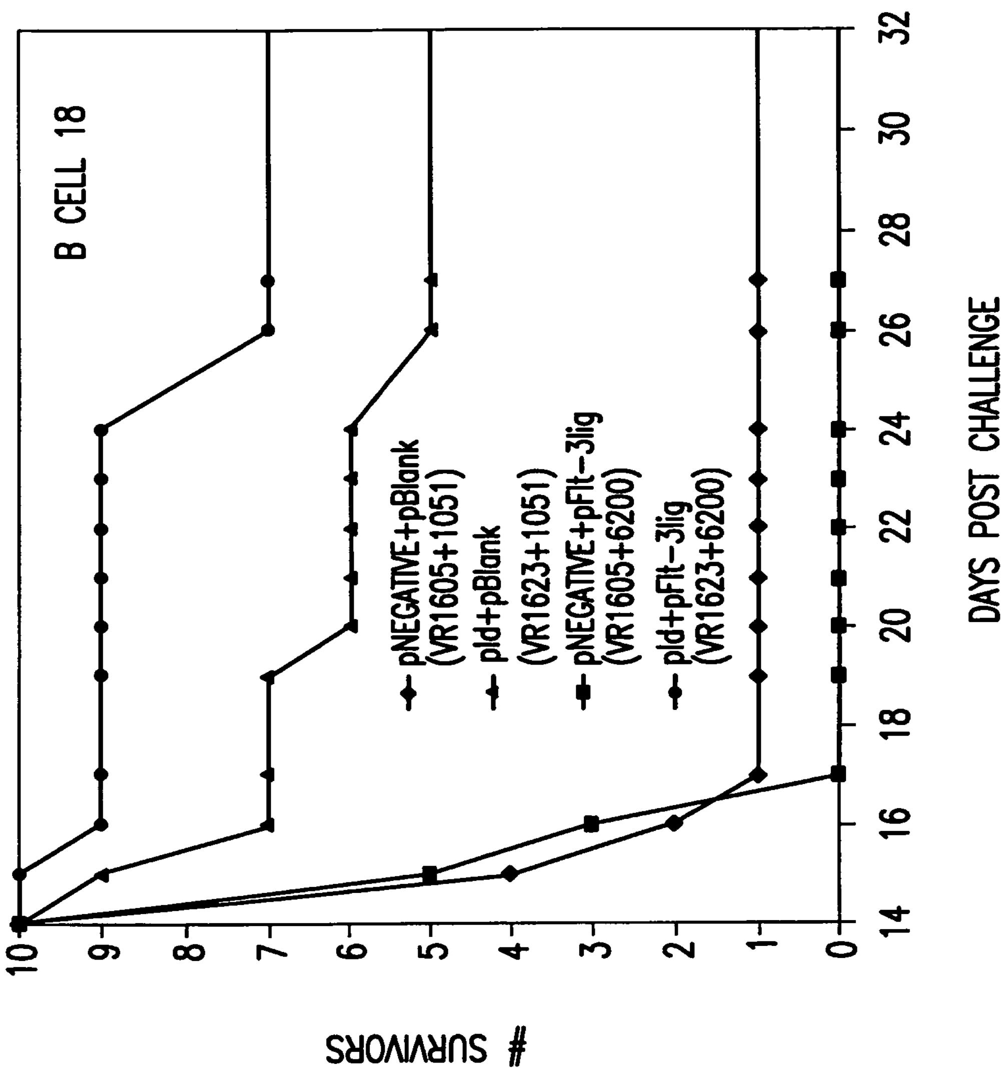
pId (VR1623)
pId+pBlank (VR1623+1051)
pId+pFlt-3lig (VR1623+6200)
Anti-Id IgG ng/ml
14000
12000
10000
8000
6000
4000
2000
0
50000
40000
30000
20000
10000
1
2
3
4
5
6
7
8
9
10

FIG.8


B CELL 18
pNEGATIVE+pBlank (VR1605+1051)
pId+pBlank (VR1623+1051)
pNEGATIVE+pFlt-3lig (VR1605+6200)
pId+pFlt-3lig (VR1623+6200)
SURVIVORS
DAYS POST CHALLENGE
0
1
2
3
4
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6
7
8
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FIG. 9

SEQUENCE LISTING

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gccaataggg actttccatt gacgtcaatg ggtggagtat ttacggtaaa ctgcccactt 180

ggcagtacat caagtgtatc atatgccaag tacgccccct attgacgtca atgacggtaa 240

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gcgtggatag cggtttgact cacggggatt tccaagtctc caccccattg acgtcaatgg 420

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tccaacatta ccgccatgtt gacattgatt attgactagt tattaatagt aatcaattac 360

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cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt tcttgaagtg 3240

gtggcctaac tacggctaca ctagaaggac agtatttggt atctgcgctc tgctgaagcc 3300

agttaccttc ggaaaaagag ttggtagctc ttgatccggc aaacaaacca ccgctggtag 3360

cggtggtttt tttgtttgca agcagcagat tacgcgcaga aaaaaaggat ctcaagaaga 3420

tcctttgatc ttttctacgg ggtctgacgc tcagtggaac gaaaactcac gttaagggat 3480

tttggtcatg agattatcaa aaaggatctt cacctagatc cttttaaatt aaaaatgaag 3540

ttttaaatca atctaaagta tatatgagta aacttggtct gacagttacc aatgcttaat 3600

cagtgaggca cctatctcag cgatctgtct atttcgttca tccatagttg cctgactccg 3660

gggggggggg gcgctgaggt ctgcctcgtg aagaaggtgt tgctgactca taccaggcct 3720

gaatcgcccc atcatccagc cagaaagtga gggagccacg gttgatgaga gctttgttgt 3780

aggtggacca gttggtgatt ttgaactttt gctttgccac ggaacggtct gcgttgtcgg 3840

gaagatgcgt gatctgatcc ttcaactcag caaaagttcg atttattcaa caaagccgcc 3900

gtcccgtcaa gtcagcgtaa tgctctgcca gtgttacaac caattaacca attctgatta 3960

gaaaaactca tcgagcatca aatgaaactg caatttattc atatcaggat tatcaatacc 4020

atatttttga aaaagccgtt tctgtaatga aggagaaaac tcaccgaggc agttccatag 4080

gatggcaaga tcctggtatc ggtctgcgat tccgactcgt ccaacatcaa tacaacctat 4140

taatttcccc tcgtcaaaaa taaggttatc aagtgagaaa tcaccatgag tgacgactga 4200

atccggtgag aatggcaaaa gcttatgcat ttctttccag acttgttcaa caggccagcc 4260

attacgctcg tcatcaaaat cactcgcatc aaccaaaccg ttattcattc gtgattgcgc 4320

ctgagcgaga cgaaatacgc gatcgctgtt aaaaggacaa ttacaaacag gaatcgaatg 4380

caaccggcgc aggaacactg ccagcgcatc aacaatattt tcacctgaat caggatattc 4440

ttctaatacc tggaatgctg ttttcccggg gatcgcagtg gtgagtaacc atgcatcatc 4500

aggagtacgg ataaaatgct tgatggtcgg aagaggcata aattccgtca gccagtttag 4560

tctgaccatc tcatctgtaa catcattggc aacgctacct ttgccatgtt tcagaaacaa 4620

ctctggcgca tcgggcttcc catacaatcg atagattgtc gcacctgatt gcccgacatt 4680

atcgcgagcc catttatacc catataaatc agcatccatg ttggaattta atcgcggcct 4740

cgagcaagac gtttcccgtt gaatatggct cataacaccc cttgtattac tgtttatgta 4800

agcagacagt tttattgttc atgatgatat atttttatct tgtgcaatgt aacatcagag 4860

attttgagac acaacgtggc tttccccccc cccccattat tgaagcattt atcagggtta 4920

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ttgtctcatg agcggataca tatttgaatg tatttagaaa aataaacaaa taggggttcc 4980

gcgcacattt ccccgaaaag tgccacctga cgtctaagaa accattatta tcatgacatt 5040

aacctataaa aataggcgta tcacgaggcc ctttcgtctc gcgcgtttcg gtgatgacgg 5100

tgaaaacctc tgacacatgc agctcccgga gacggtcaca gcttgtctgt aagcggatgc 5160

cgggagcaga caagcccgtc agggcgcgtc agcgggtgtt ggcgggtgtc ggggctggct 5220

taactatgcg gcatcagagc agattgtact gagagtgcac catatgcggt gtgaaatacc 5280

gcacagatgc gtaaggagaa aataccgcat cagattggct at                    5322


<210> 7
<211> 829
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (32)..(724)

<220>
<221> CDS
<222> (32)..(598)

<400> 7
ctgtcacagg catgaggggt ccccggcaga g atg aca gtg ctg gcg cca gcc      52
                                   Met Thr Val Leu Ala Pro Ala
                                    1               5

tgg agc cca aat tcc tcc ctg ttg ctg ctg ttg ctg ctg ctg agt cct     100
Trp Ser Pro Asn Ser Ser Leu Leu Leu Leu Leu Leu Leu Leu Ser Pro
        10                  15                  20

tgc ctg cgg ggg aca cct gac tgt tac ttc agc cac agt ccc atc tcc     148
Cys Leu Arg Gly Thr Pro Asp Cys Tyr Phe Ser His Ser Pro Ile Ser
    25                  30                  35

tcc aac ttc aaa gtg aag ttt aga gag ttg act gac cac ctg ctt aaa     196
Ser Asn Phe Lys Val Lys Phe Arg Glu Leu Thr Asp His Leu Leu Lys
 40                  45                  50                  55

gat tac cca gtc act gtg gcc gtc aat ctt cag gac gag aag cac tgc     244
Asp Tyr Pro Val Thr Val Ala Val Asn Leu Gln Asp Glu Lys His Cys
                 60                  65                  70

aag gcc ttg tgg agc ctc ttc cta gcc cag cgc tgg ata gag caa ctg     292
Lys Ala Leu Trp Ser Leu Phe Leu Ala Gln Arg Trp Ile Glu Gln Leu
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            75                  80                  85

aag act gtg gca ggg tct aag atg caa acg ctt ctg gag gac gtc aac    340
Lys Thr Val Ala Gly Ser Lys Met Gln Thr Leu Leu Glu Asp Val Asn
        90                  95                  100

acc gag ata cat ttt gtc acc tca tgt acc ttc cag ccc cta cca gaa    388
Thr Glu Ile His Phe Val Thr Ser Cys Thr Phe Gln Pro Leu Pro Glu
    105                 110                 115

tgt ctg cga ttc gtc cag acc aac atc tcc cac ctc ctg aag gac acc    436
Cys Leu Arg Phe Val Gln Thr Asn Ile Ser His Leu Leu Lys Asp Thr
120                 125                 130                 135

tgc aca cag ctg ctt gct ctg aag ccc tgt atc ggg aag gcc tgc cag    484
Cys Thr Gln Leu Leu Ala Leu Lys Pro Cys Ile Gly Lys Ala Cys Gln
                140                 145                 150

aat ttc tct cgg tgc ctg gag gtg cag tgc cag ccg gac tcc tcc acc    532
Asn Phe Ser Arg Cys Leu Glu Val Gln Cys Gln Pro Asp Ser Ser Thr
            155                 160                 165

ctg ctg ccc cca agg agt ccc ata gcc cta gaa gcc acg gag ctc cca    580
Leu Leu Pro Pro Arg Ser Pro Ile Ala Leu Glu Ala Thr Glu Leu Pro
        170                 175                 180

gag cct cgg ccc agg cag ctg ttg ctc ctg ctg ctg ctg ctg cct ctc    628
Glu Pro Arg Pro Arg Gln Leu Leu Leu Leu Leu Leu Leu Leu Pro Leu
    185                 190                 195

aca ctg gtg ctg ctg gca gcc gcc tgg ggc ctt cgc tgg caa agg gca    676
Thr Leu Val Leu Leu Ala Ala Ala Trp Gly Leu Arg Trp Gln Arg Ala
200                 205                 210                 215

aga agg agg ggg gag ctc cac cct ggg gtg ccc ctc ccc tcc cat ccc    724
Arg Arg Arg Gly Glu Leu His Pro Gly Val Pro Leu Pro Ser His Pro
                220                 225                 230

ctgttgctcc tgctgctgct gctgcctctc acactggtgc tgctggcagc             774

cgcctggggc cttcgctggc aaagggcaag aaggaggggg gagctccacc             824

ctggggtgcc cctcccctcc catccctagg attcgagcct tgtgcatcgt             874

tgactcagcc agggtcttat ctcggttaca cctgtaatct cagcccttgg             924

gagcccagag caggattgct gaatggtctg g                                 955
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<210> 8
<211> 231
<212> PRT

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<213> Mus musculus

<400> 8
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
  1               5                  10                  15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
             20                  25                  30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
         35                  40                  45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
     50                  55                  60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
 65                  70                  75                  80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
                 85                  90                  95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
            100                 105                 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
        115                 120                 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
    130                 135                 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145                 150                 155                 160

Cys Gln Pro Asp Ser Ser Thr Leu Leu Pro Pro Arg Ser Pro Ile Ala
                165                 170                 175

Leu Glu Ala Thr Glu Leu Pro Glu Pro Arg Pro Arg Gln Leu Leu Leu
            180                 185                 190

Leu Leu Leu Leu Leu Pro Leu Thr Leu Val Leu Leu Ala Ala Ala Trp
        195                 200                 205

Gly Leu Arg Trp Gln Arg Ala Arg Arg Arg Gly Glu Leu His Pro Gly
    210                 215                 220

Val Pro Leu Pro Ser His Pro
225                 230


<210> 9
<211> 189
<212> PRT
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<213> Mus musculus

<400> 9
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
  1               5                  10                  15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
             20                  25                  30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
         35                  40                  45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
     50                  55                  60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
 65                  70                  75                  80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
                 85                  90                  95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
            100                 105                 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
        115                 120                 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
    130                 135                 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145                 150                 155                 160

Cys Gln Pro Asp Ser Ser Thr Leu Leu Pro Pro Arg Ser Pro Ile Ala
                165                 170                 175

Leu Glu Ala Thr Glu Leu Pro Glu Pro Arg Pro Arg Gln
            180                 185

<210> 10
<211> 852
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (7)..(666)

<400> 10
gcagag atg aca gtg ctg gcg cca gcc tgg agc cca aat tcc tcc ctg       48
       Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu
         1               5                  10
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ttg ctg ctg ttg ctg ctg ctg agt cct tgc ctg cgg ggg aca cct gac    96
Leu Leu Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp
 15                 20                  25                  30

tgt tac ttc agc cac agt ccc atc tcc tcc aac ttc aaa gtg aag ttt   144
Cys Tyr Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe
                35                  40                  45

aga gag ttg act gac cac ctg ctt aaa gat tac cca gtc act gtg gcc   192
Arg Glu Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala
            50                  55                  60

gtc aat ctt cag gac gag aag cac tgc aag gcc ttg tgg agc ctc ttc   240
Val Asn Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe
        65                  70                  75

cta gcc cag cgc tgg ata gag caa ctg aag act gtg gca ggg tct aag   288
Leu Ala Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys
    80                  85                  90

atg caa acg ctt ctg gag gac gtc aac acc gag ata cat ttt gtc acc   336
Met Gln Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr
 95                 100                 105                 110

tca tgt acc ttc cag ccc cta cca gaa tgt ctg cga ttc gtc cag acc   384
Ser Cys Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr
                115                 120                 125

aac atc tcc cac ctc ctg aag gac acc tgc aca cag ctg ctt gct ctg   432
Asn Ile Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu
            130                 135                 140

aag ccc tgt atc ggg aag gcc tgc cag aat ttc tct cgg tgc ctg gag   480
Lys Pro Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu
        145                 150                 155

gtg cag tgc cag ccg ggt aac ggt ggc ccc aga gcc cag cac cat ggt   528
Val Gln Cys Gln Pro Gly Asn Gly Gly Pro Arg Ala Gln His His Gly
    160                 165                 170

gcc acc agg ctc aca gcc aca gcc ttg cta act gtg tgt cca ggg ctt   576
Ala Thr Arg Leu Thr Ala Thr Ala Leu Leu Thr Val Cys Pro Gly Leu
175                 180                 185                 190

ctg ctc cca cta gtt ggc act tca cac atg ttc ttt ctc cct tat ttt   624
Leu Leu Pro Leu Val Gly Thr Ser His Met Phe Phe Leu Pro Tyr Phe
                195                 200                 205

ctc tct ttt ctt tct tct ttt tta aag atg tat ctt tat gtg           666
Leu Ser Phe Leu Ser Ser Phe Leu Lys Met Tyr Leu Tyr Val
            210                 215                 220
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tgagtgtttt acctacatgc ctgtaagtgc actgaatgtg tgtctggtgc ctgcagaggc 726

cagaagaggg caccagatcc cctgaaactg gagtctctgc tccgtgtgaa ccaccacgtg 786

ggtgctggga cccaggtcca atgcaagagc acccagggtt cttacctgct gagccaccac 846

ttcaac 852

<210> 11
<211> 220
<212> PRT
<213> Mus musculus

<400> 11
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
1 5 10 15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
20 25 30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
35 40 45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
50 55 60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
65 70 75 80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
85 90 95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
100 105 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
115 120 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
130 135 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145 150 155 160

Cys Gln Pro Gly Asn Gly Gly Pro Arg Ala Gln His His Gly Ala Thr
165 170 175

Arg Leu Thr Ala Thr Ala Leu Leu Thr Val Cys Pro Gly Leu Leu Leu
180 185 190

```
Pro Leu Val Gly Thr Ser His Met Phe Phe Leu Pro Tyr Phe Leu Ser
        195                 200                 205

Phe Leu Ser Ser Phe Leu Lys Met Tyr Leu Tyr Val
    210                 215                 220


<210> 12
<211> 1152
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (256)..(951)

<400> 12
gaattcgcgg ccgcgtcgac attctgggga cgtcggtcgg ggttcttaga agaggagatg 60

acttttcaca gtcactgagg ctcgtgcagg aagcctgggg gagcaggagg cggaaaccga 120

cccacatcaa gggcggcagg gccgggcggc ggggtacagg ggttgggggg gaaggggctg 180

cagggtatga gcccgagacc tgccctcctg tcacttccaa gaacctgtca caggcatgag 240

gggtccccgg cagag atg aca gtg ctg gcg cca gcc tgg agc cca aat tcc  291
                 Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser
                  1               5                  10

tcc ctg ttg ctg ctg ttg ctg ctg ctg agt cct tgc ctg cgg ggg aca   339
Ser Leu Leu Leu Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr
        15                  20                  25

cct gac tgt tac ttc agc cac agt ccc atc tcc tcc aac ttc aaa gtg   387
Pro Asp Cys Tyr Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val
    30                  35                  40

aag ttt aga gag ttg act gac cac ctg ctt aaa gat tac cca gtc act   435
Lys Phe Arg Glu Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr
45                  50                  55                  60

gtg gcc gtc aat ctt cag gac gag aag cac tgc aag gcc ttg tgg agc   483
Val Ala Val Asn Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser
                65                  70                  75

ctc ttc cta gcc cag cgc tgg ata gag caa ctg aag act gtg gca ggg   531
Leu Phe Leu Ala Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly
            80                  85                  90

tct aag atg caa acg ctt ctg gag gac gtc aac acc gag ata cat ttt   579
Ser Lys Met Gln Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe
        95                  100                 105
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gtc acc tca tgt acc ttc cag ccc cta cca gaa tgt ctg cga ttc gtc 627
Val Thr Ser Cys Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val
110 115 120

cag acc aac atc tcc cac ctc ctg aag gac acc tgc aca cag ctg ctt 675
Gln Thr Asn Ile Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu
125 130 135 140

ggt ctg aag ccc tgt atc ggg aag gcc tgc cag aat ttc tct cgg tgc 723
Gly Leu Lys Pro Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys
145 150 155

ctg gag gtg cag tgc cag ccg gac tcc tcc acc ctg ctg ccc cca agg 771
Leu Glu Val Gln Cys Gln Pro Asp Ser Ser Thr Leu Leu Pro Pro Arg
160 165 170

agt ccc ata gcc cta gaa gcc acg gag ctc cca gag cct cgg ccc agg 819
Ser Pro Ile Ala Leu Glu Ala Thr Glu Leu Pro Glu Pro Arg Pro Arg
175 180 185

cag ctg ttg ctc ctg ctg ctg ctg ctg ctg cct ctc aca ctg gtg ctg 867
Gln Leu Leu Leu Leu Leu Leu Leu Leu Leu Pro Leu Thr Leu Val Leu
190 195 200

ctg gca gcc gcc tgg ggc ctt cgc tgg caa agg gca aga agg agg ggg 915
Leu Ala Ala Ala Trp Gly Leu Arg Trp Gln Arg Ala Arg Arg Arg Gly
205 210 215 220

gag ctc cac cct ggg gtg ccc ctc ccc tcc cat ccc taggatgcga 961
Glu Leu His Pro Gly Val Pro Leu Pro Ser His Pro
225 230

gccttgtgca tcgttgactc agccagggtc ttatctcgat gaggtctcaa tatgttgccc 1021

aaactgactt tgaaaacctc gatgcacctt cctgccccac aaacttccaa acagctgggc 1081

ttacgggcat gctatataca acaaggcttt cttttcttct ttcttggtgc tagagttggg 1141

aaccaaaaca a 1152

<210> 13
<211> 232
<212> PRT
<213> Mus musculus

<400> 13
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
1 5 10 15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr

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            20                  25                  30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
        35                  40                  45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
    50                  55                  60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
65                  70                  75                  80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
                85                  90                  95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
            100                 105                 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
        115                 120                 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Gly Leu Lys Pro
    130                 135                 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145                 150                 155                 160

Cys Gln Pro Asp Ser Ser Thr Leu Leu Pro Pro Arg Ser Pro Ile Ala
                165                 170                 175

Leu Glu Ala Thr Glu Leu Pro Glu Pro Arg Pro Arg Gln Leu Leu Leu
            180                 185                 190

Leu Leu Leu Leu Leu Leu Pro Leu Thr Leu Val Leu Leu Ala Ala Ala
        195                 200                 205

Trp Gly Leu Arg Trp Gln Arg Ala Arg Arg Arg Gly Glu Leu His Pro
    210                 215                 220

Gly Val Pro Leu Pro Ser His Pro
225                 230


<210> 14
<211> 663
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(660)

<400> 14
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atg aca gtg ctg gcg cca gcc tgg agc cca aat tcc tcc ctg ttg ctg    48
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
  1               5                  10                  15

ctg ttg ctg ctg ctg agt cct tgc ctg cgg ggg aca cct gac tgt tac    96
Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
             20                  25                  30

ttc agc cac agt ccc atc tcc tcc aac ttc aaa gtg aag ttt aga gag   144
Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
         35                  40                  45

ttg act gac cac ctg ctt aaa gat tac cca gtc act gtg gcc gtc aat   192
Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
     50                  55                  60

ctt cag gac gag aag cac tgc aag gcc ttg tgg agc ctc ttc cta gcc   240
Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
 65                  70                  75                  80

cag cgc tgg ata gag caa ctg aag act gtg gca ggg tct aag atg caa   288
Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
                 85                  90                  95

acg ctt ctg gag gac gtc aac acc gag ata cat ttt gtc acc tca tgt   336
Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
            100                 105                 110

acc ttc cag ccc cta cca gaa tgt ctg cga ttc gtc cag acc aac atc   384
Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
        115                 120                 125

tcc cac ctc ctg aag gac acc tgc aca cag ctg ctt gct ctg aag ccc   432
Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
    130                 135                 140

tgt atc ggg aag gcc tgc cag aat ttc tct cgg tgc ctg gag gtg cag   480
Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145                 150                 155                 160

tgc cag ccg ggt aac ggt ggc ccc aga gcc cag cac cat ggt gcc acc   528
Cys Gln Pro Gly Asn Gly Gly Pro Arg Ala Gln His His Gly Ala Thr
                165                 170                 175

agg ctc aca gcc aca gcc ttg cta act gtg tgt cca ggg ctt ctg ctc   576
Arg Leu Thr Ala Thr Ala Leu Leu Thr Val Cys Pro Gly Leu Leu Leu
            180                 185                 190

cca cta gtt ggc act tca cac atg ttc ttt ctc cct tat ttt ctc tct   624
Pro Leu Val Gly Thr Ser His Met Phe Phe Leu Pro Tyr Phe Leu Ser
        195                 200                 205
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ttt ctt tct tct ttt tta aag atg tat ctt tat gtg tga 663
Phe Leu Ser Ser Phe Leu Lys Met Tyr Leu Tyr Val
210 215 220

<210> 15
<211> 220
<212> PRT
<213> Mus musculus

<400> 15
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
1 5 10 15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
20 25 30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
35 40 45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
50 55 60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
65 70 75 80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
85 90 95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
100 105 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
115 120 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
130 135 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145 150 155 160

Cys Gln Pro Gly Asn Gly Gly Pro Arg Ala Gln His His Gly Ala Thr
165 170 175

Arg Leu Thr Ala Thr Ala Leu Leu Thr Val Cys Pro Gly Leu Leu Leu
180 185 190

Pro Leu Val Gly Thr Ser His Met Phe Phe Leu Pro Tyr Phe Leu Ser
195 200 205

Phe Leu Ser Ser Phe Leu Lys Met Tyr Leu Tyr Val
210 215 220

<210> 16
<211> 519
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(516)

<400> 16
atg aca gtg ctg gcg cca gcc tgg agc cca aat tcc tcc ctg ttg ctg 48
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
1 5 10 15

ctg ttg ctg ctg ctg agt cct tgc ctg cgg ggg aca cct gac tgt tac 96
Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
20 25 30

ttc agc cac agt ccc atc tcc tcc aac ttc aaa gtg aag ttt aga gag 144
Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
35 40 45

ttg act gac cac ctg ctt aaa gat tac cca gtc act gtg gcc gtc aat 192
Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
50 55 60

ctt cag gac gag aag cac tgc aag gcc ttg tgg agc ctc ttc cta gcc 240
Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
65 70 75 80

cag cgc tgg ata gag caa ctg aag act gtg gca ggg tct aag atg caa 288
Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
85 90 95

acg ctt ctg gag gac gtc aac acc gag ata cat ttt gtc acc tca tgt 336
Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
100 105 110

acc ttc cag ccc cta cca gaa tgt ctg cga ttc gtc cag acc aac atc 384
Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
115 120 125

tcc cac ctc ctg aag gac acc tgc aca cag ctg ctt gct ctg aag ccc 432
Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
130 135 140

tgt atc ggg aag gcc tgc cag aat ttc tct cgg tgc ctg gag gtg cag 480
Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145 150 155 160

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tgc cag ccg gat agg gtc tca tta tgc agg cta ggc tga                519
Cys Gln Pro Asp Arg Val Ser Leu Cys Arg Leu Gly
                165                 170


<210> 17
<211> 172
<212> PRT
<213> Mus musculus

<400> 17
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
  1               5                  10                  15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
             20                  25                  30

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
         35                  40                  45

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
     50                  55                  60

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
 65                  70                  75                  80

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
                 85                  90                  95

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
            100                 105                 110

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
        115                 120                 125

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
    130                 135                 140

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
145                 150                 155                 160

Cys Gln Pro Asp Arg Val Ser Leu Cys Arg Leu Gly
                165                 170


<210> 18
<211> 1080
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
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<222> (84)..(788)

<400> 18
ccggggggca tgagggtccg agacttgttc ttctgtccct tccaagaccc ggcgacagga 60

ggcatgaggg gcccccggcc gaa atg aca gtg ctg gcg cca gcc tgg agc cca 113
Met Thr Val Leu Ala Pro Ala Trp Ser Pro
1 5 10

aca acc tat ctc ctc ctg ctg ctg ctg ctg agc tcg gga ctc agt ggg 161
Thr Thr Tyr Leu Leu Leu Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly
15 20 25

acc cag gac tgc tcc ttc caa cac agc ccc atc tcc tcc gac ttc gct 209
Thr Gln Asp Cys Ser Phe Gln His Ser Pro Ile Ser Ser Asp Phe Ala
30 35 40

gtc aaa atc cgt gag ctg tct gac tac ctg ctt caa gat tac cca gtc 257
Val Lys Ile Arg Glu Leu Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val
45 50 55

acc gtg gcc tcc aac ctg cag gac gag gag ctc tgc ggg ggc ctc tgg 305
Thr Val Ala Ser Asn Leu Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp
60 65 70

cgg ctg gtc ctg gca cag cgc tgg atg gag cgg ctc aag act gtc gct 353
Arg Leu Val Leu Ala Gln Arg Trp Met Glu Arg Leu Lys Thr Val Ala
75 80 85 90

ggg tcc aag atg caa ggc ttg ctg gag cgc gtg aac acg gag ata cac 401
Gly Ser Lys Met Gln Gly Leu Leu Glu Arg Val Asn Thr Glu Ile His
95 100 105

ttt gtc acc aaa tgt gcc ttt cag ccc ccc ccc agc tgt ctt cgc ttc 449
Phe Val Thr Lys Cys Ala Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe
110 115 120

gtc cag acc aac atc tcc cgc ctc ctg cag gag acc tcc gag cag ctg 497
Val Gln Thr Asn Ile Ser Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu
125 130 135

gtg gcg ctg aag ccc tgg atc act cgc cag aac ttc tcc cgg tgc ctg 545
Val Ala Leu Lys Pro Trp Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu
140 145 150

gag ctg cag tgt cag ccc gac tcc tca acc ctg cca ccc cca tgg agt 593
Glu Leu Gln Cys Gln Pro Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser
155 160 165 170

ccc cgg ccc ctg gag gcc aca gcc ccg aca gcc ccg cag ccc cct ctg 641
Pro Arg Pro Leu Glu Ala Thr Ala Pro Thr Ala Pro Gln Pro Pro Leu
175 180 185

```
ctc ctc cta ctg ctg ctg ccc gtg ggc ctc ctg ctg ctg gcc gct gcc    689
Leu Leu Leu Leu Leu Leu Pro Val Gly Leu Leu Leu Leu Ala Ala Ala
            190                 195                 200

tgg tgc ctg cac tgg cag agg acg cgg cgg agg aca ccc cgc cct ggg    737
Trp Cys Leu His Trp Gln Arg Thr Arg Arg Arg Thr Pro Arg Pro Gly
        205                 210                 215

gag cag gtg ccc ccc gtc ccc agt ccc cag gac ctg ctg ctt gtg gag    785
Glu Gln Val Pro Pro Val Pro Ser Pro Gln Asp Leu Leu Leu Val Glu
    220                 225                 230

cac tgacctggcc aaggcctcat cctgcggagc cttaaacaac gcagtgagac         838
His
235

agacatctat catcccattt tacaggggag gatactgagg cacacagagg ggagtcacca 898

gccagaggat gtatagcctg gacacagagg aagttggcta gaggccggtc ccttccttgg 958

gcccctctca ttccctcccc agaatggagg caacgccaga atccagcacc ggccccattt 1018

acccaactct gaacaaagcc cttgccccca tgaaattgtt tataaatcat ccttttctcc 1078

ca                                                                1080


<210> 19
<211> 235
<212> PRT
<213> Homo sapiens

<400> 19
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Thr Thr Tyr Leu Leu Leu
 1               5                  10                  15

Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly Thr Gln Asp Cys Ser Phe
            20                  25                  30

Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
        35                  40                  45

Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu
    50                  55                  60

Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu Ala Gln
65                  70                  75                  80

Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
                85                  90                  95
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Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
100 105 110

Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
115 120 125

Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
130 135 140

Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
145 150 155 160

Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu Glu Ala
165 170 175

Thr Ala Pro Thr Ala Pro Gln Pro Pro Leu Leu Leu Leu Leu Leu Leu
180 185 190

Pro Val Gly Leu Leu Leu Leu Ala Ala Ala Trp Cys Leu His Trp Gln
195 200 205

Arg Thr Arg Arg Arg Thr Pro Arg Pro Gly Glu Gln Val Pro Pro Val
210 215 220

Pro Ser Pro Gln Asp Leu Leu Leu Val Glu His
225 230 235

<210> 20
<211> 537
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(537)

<400> 20
atg aca gtg ctg gcg cca gcc tgg agc cca aca acc tat ctc ctc ctg 48
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Thr Thr Tyr Leu Leu Leu
1 5 10 15

ctg ctg ctg ctg agc tcg gga ctc agt ggg acc cag gac tgc tcc ttc 96
Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly Thr Gln Asp Cys Ser Phe
20 25 30

caa cac agc ccc atc tcc tcc gac ttc gct gtc aaa atc cgt gag ctg 144
Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
35 40 45

tct gac tac ctg ctt caa gat tac cca gtc acc gtg gcc tcc aac ctg 192
Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu

```
    50                  55                  60

cag gac gag gag ctc tgc ggg ggc ctc tgg cgg ctg gtc ctg gca cag      240
Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu Ala Gln
 65                  70                  75                  80

cgc tgg atg gag cgg ctc aag act gtc gct ggg tcc aag atg caa ggc      288
Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
                 85                  90                  95

ttg ctg gag cgc gtg aac acg gag ata cac ttt gtc acc aaa tgt gcc      336
Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
            100                 105                 110

ttt cag ccc ccc ccc agc tgt ctt cgc ttc gtc cag acc aac atc tcc      384
Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
        115                 120                 125

cgc ctc ctg cag gag acc tcc gag cag ctg gtg gcg ctg aag ccc tgg      432
Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
    130                 135                 140

atc act cgc cag aac ttc tcc cgg tgc ctg gag ctg cag tgt cag ccc      480
Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
145                 150                 155                 160

gta gag acg gtg ttt cac cgt gtc agc cag gat ggt ctc gat ctc ctg      528
Val Glu Thr Val Phe His Arg Val Ser Gln Asp Gly Leu Asp Leu Leu
                165                 170                 175

acc tcg tga                                                          537
Thr Ser


&lt;210&gt; 21
&lt;211&gt; 178
&lt;212&gt; PRT
&lt;213&gt; Homo sapiens

&lt;400&gt; 21
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Thr Thr Tyr Leu Leu Leu
  1               5                  10                  15

Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly Thr Gln Asp Cys Ser Phe
             20                  25                  30

Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
         35                  40                  45

Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu
     50                  55                  60
```

```
Gln Asp Glu Glu Leu Cys Gly Gly Leu Trp Arg Leu Val Leu Ala Gln
 65                 70                  75                  80

Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
                85                  90                  95

Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
            100                 105                 110

Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
        115                 120                 125

Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
    130                 135                 140

Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
145                 150                 155                 160

Val Glu Thr Val Phe His Arg Val Ser Gln Asp Gly Leu Asp Leu Leu
                165                 170                 175

Thr Ser


<210> 22
<211> 859
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (93)..(797)

<220>
<221> CDS
<222> (93)..(647)

<400> 22
tggaaagggc tgtcacccgg cttggcccct tccacaccca actggggcaa gcctgacccg 60

gcgacaggag gcatgagggg cccccggccg aa atg aca gtg ctg gcg cca gcc    113
                                    Met Thr Val Leu Ala Pro Ala
                                    1               5

tgg agc cca aca acc tat ctc ctc ctg ctg ctg ctg ctg agc tcg gga    161
Trp Ser Pro Thr Thr Tyr Leu Leu Leu Leu Leu Leu Leu Ser Ser Gly
        10                  15                  20

ctc agt ggg acc cag gac tgc tcc ttc caa cac agc ccc atc tcc tcc    209
Leu Ser Gly Thr Gln Asp Cys Ser Phe Gln His Ser Pro Ile Ser Ser
    25                  30                  35
```

```
gac ttc gct gtc aaa atc cgt gag ctg tct gac tac ctg ctt caa gat     257
Asp Phe Ala Val Lys Ile Arg Glu Leu Ser Asp Tyr Leu Leu Gln Asp
 40                  45                  50                  55

tac cca gtc acc gtg gcc tcc aac ctg cag gac gag gag ctc tgc ggg     305
Tyr Pro Val Thr Val Ala Ser Asn Leu Gln Asp Glu Glu Leu Cys Gly
                 60                  65                  70

gcg ctc tgg cgg ctg gtc ctg gca cag cgc tgg atg gag cgg ctc aag     353
Ala Leu Trp Arg Leu Val Leu Ala Gln Arg Trp Met Glu Arg Leu Lys
             75                  80                  85

act gtc gct ggg tcc aag atg caa ggc ttg ctg gag cgc gtg aac acg     401
Thr Val Ala Gly Ser Lys Met Gln Gly Leu Leu Glu Arg Val Asn Thr
         90                  95                 100

gag ata cac ttt gtc acc aaa tgt gcc ttt cag ccc ccc ccc agc tgt     449
Glu Ile His Phe Val Thr Lys Cys Ala Phe Gln Pro Pro Pro Ser Cys
    105                 110                 115

ctt cgc ttc gtc cag acc aac atc tcc cgc ctc ctg cag gag acc tcc     497
Leu Arg Phe Val Gln Thr Asn Ile Ser Arg Leu Leu Gln Glu Thr Ser
120                 125                 130                 135

gag cag ctg gtg gcg ctg aag ccc tgg atc act cgc cag aac ttc tcc     545
Glu Gln Leu Val Ala Leu Lys Pro Trp Ile Thr Arg Gln Asn Phe Ser
                140                 145                 150

cgg tgc ctg gag ctg cag tgt cag ccc gac tcc tca acc ctg cca ccc     593
Arg Cys Leu Glu Leu Gln Cys Gln Pro Asp Ser Ser Thr Leu Pro Pro
            155                 160                 165

cca tgg agt ccc cgg ccc ctg gag gcc aca gcc ccg aca gcc ccg cag     641
Pro Trp Ser Pro Arg Pro Leu Glu Ala Thr Ala Pro Thr Ala Pro Gln
        170                 175                 180

ccc cct ctg ctc ctc cta ctg ctg ctg ccc gtg ggc ctc ctg ctg ctg     689
Pro Pro Leu Leu Leu Leu Leu Leu Leu Pro Val Gly Leu Leu Leu Leu
    185                 190                 195

gcc gct gcc tgg tgc ctg cac tgg cag agg acg cgg cgg agg aca ccc     737
Ala Ala Ala Trp Cys Leu His Trp Gln Arg Thr Arg Arg Arg Thr Pro
200                 205                 210                 215

cgc cct ggg gag cag gtg ccc ccc gtc ccc agt ccc cag gac ctg ctg     785
Arg Pro Gly Glu Gln Val Pro Pro Val Pro Ser Pro Gln Asp Leu Leu
                220                 225                 230

ctt gtg gag cac  ctgctcctcc tactgctgct gcccgtgggc ctcctgctgc        837
Leu Val Glu His
            235
```

tggccgctgc ctggtgcctg cactggcaga ggacgcggcg gaggacaccc cgccctgggg 897

agcaggtgcc ccccgtcccc agtccccagg acctgctgct tgtggagcac tgacctggcc 957

aaggcctcat cctggggagg atacgtaggc acacagaggg gagtcaccag cc 1009

<210> 23
<211> 235
<212> PRT
<213> Homo sapiens

<400> 23
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Thr Thr Tyr Leu Leu Leu
1 5 10 15

Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly Thr Gln Asp Cys Ser Phe
20 25 30

Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
35 40 45

Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu
50 55 60

Gln Asp Glu Glu Leu Cys Gly Ala Leu Trp Arg Leu Val Leu Ala Gln
65 70 75 80

Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
85 90 95

Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
100 105 110

Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
115 120 125

Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
130 135 140

Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
145 150 155 160

Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu Glu Ala
165 170 175

Thr Ala Pro Thr Ala Pro Gln Pro Pro Leu Leu Leu Leu Leu Leu Leu
180 185 190

Pro Val Gly Leu Leu Leu Leu Ala Ala Ala Trp Cys Leu His Trp Gln
195 200 205

```
Arg Thr Arg Arg Arg Thr Pro Arg Pro Gly Glu Gln Val Pro Pro Val
    210                 215                 220

Pro Ser Pro Gln Asp Leu Leu Leu Val Glu His
225                 230                 235


<210> 24
<211> 185
<212> PRT
<213> Homo sapiens

<400> 24
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Thr Thr Tyr Leu Leu Leu
  1               5                  10                  15

Leu Leu Leu Leu Ser Ser Gly Leu Ser Gly Thr Gln Asp Cys Ser Phe
             20                  25                  30

Gln His Ser Pro Ile Ser Ser Asp Phe Ala Val Lys Ile Arg Glu Leu
         35                  40                  45

Ser Asp Tyr Leu Leu Gln Asp Tyr Pro Val Thr Val Ala Ser Asn Leu
     50                  55                  60

Gln Asp Glu Glu Leu Cys Gly Ala Leu Trp Arg Leu Val Leu Ala Gln
 65                  70                  75                  80

Arg Trp Met Glu Arg Leu Lys Thr Val Ala Gly Ser Lys Met Gln Gly
                 85                  90                  95

Leu Leu Glu Arg Val Asn Thr Glu Ile His Phe Val Thr Lys Cys Ala
            100                 105                 110

Phe Gln Pro Pro Pro Ser Cys Leu Arg Phe Val Gln Thr Asn Ile Ser
        115                 120                 125

Arg Leu Leu Gln Glu Thr Ser Glu Gln Leu Val Ala Leu Lys Pro Trp
    130                 135                 140

Ile Thr Arg Gln Asn Phe Ser Arg Cys Leu Glu Leu Gln Cys Gln Pro
145                 150                 155                 160

Asp Ser Ser Thr Leu Pro Pro Pro Trp Ser Pro Arg Pro Leu Glu Ala
                165                 170                 175

Thr Ala Pro Thr Ala Pro Gln Pro Pro
            180                 185

<210> 25
<211> 36
<212> DNA
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 25
cacgaattcg ccgccaccat gacagtgctg gcgcca 36

<210> 26
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 26
gccgctagct cactgcctgg gccgaggctc t 31

<210> 27
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 27
aaaactgcag gccttgctgg ccacgcgtta ttttccacca tatt 44

<210> 28
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 28
cgcggatccg gccgctgcgg ccatggtatt atcatc 36

<210> 29
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 29
cgacgcgtgt ggctgcacca tctgtctggc ccttccggcc atctgatgag cagttg 56

<210> 30
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 30
cgacgcgttc aacactctcc cctgttgaag ct 32

<210> 31
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 31
ggaagatcta ccaagggccc atcgggcctc tccccctggc accctcc 47

<210> 32
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 32
ggaagatctc aggatctcgg agacagggag aggct 35

<210> 33
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 33
ctgcagaagg ccttgctggc cgccatggga ccgtctgttc agttc 45

<210> 34
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 34
tggtgcggcc accgtggcct ttatttccaa ctt 33

<210> 35
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 35
ctgcagaagg ccgcagcggc cgagttgtgg ctgaactgga ttttc 45

<210> 36
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 36
ccgatgggcc cttggggcca gctgaggaga cggt 34

<210> 37
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 37
gcggaattcg ccgccaccat gacagtgctg gcgccagc 38

<210> 38
<211> 29
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: DNA Primer

<400> 38
gcggctagct caaggggct gcggggctg 29

<210> 39
<211> 5310
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cDNA

<400> 39
tggccattgc atacgttgta tccatatcat aatatgtaca tttatattgg ctcatgtcca 60

acattaccgc catgttgaca ttgattattg actagttatt aatagtaatc aattacgggg 120

tcattagttc atagcccata tatggagttc cgcgttacat aacttacggt aaatggcccg 180

cctggctgac cgcccaacga cccccgccca ttgacgtcaa taatgacgta tgttcccata 240

gtaacgccaa tagggacttt ccattgacgt caatgggtgg agtatttacg gtaaactgcc 300

cacttggcag tacatcaagt gtatcatatg ccaagtacgc cccctattga cgtcaatgac 360

ggtaaatggc ccgcctggca ttatgcccag tacatgacct tatgggactt tcctacttgg 420

cagtacatct acgtattagt catcgctatt accatggtga tgcggttttg gcagtacatc 480

aatgggcgtg gatagcggtt tgactcacgg ggatttccaa gtctccaccc cattgacgtc 540

aatgggagtt tgttttggca ccaaaatcaa cgggactttc caaaatgtcg taacaactcc 600

gccccattga cgcaaatggg cggtaggcgt gtacggtggg aggtctatat aagcagagct 660

cgtttagtga accgtcagat cgcctggaga cgccatccac gctgttttga cctccataga 720

agacaccggg accgatccag cctccgcggc cgggaacggt gcattggaac gcggattccc 780

cgtgccaaga gtgacgtaag taccgcctat agactctata ggcacacccc tttggctctt 840

atgcatgcta tactgttttt ggcttggggc ctatacaccc ccgcttcctt atgctatagg 900

tgatggtata gcttagccta taggtgtggg ttattgacca ttattgacca ctcccctatt 960

ggtgacgata ctttccatta ctaatccata acatggctct ttgccacaac tatctctatt 1020

ggctatatgc caatactctg tccttcagag actgacacgg actctgtatt tttacaggat 1080

ggggtcccat ttattattta caaattcaca tatacaacaa cgccgtcccc cgtgcccgca 1140

gtttttatta aacatagcgt gggatctcca cgcgaatctc gggtacgtgt tccggacatg 1200

ggctcttctc cggtagcggc ggagcttcca catccgagcc ctggtcccat gcctccagcg 1260

gctcatggtc gctcggcagc tccttgctcc taacagtgga ggccagactt aggcacagca 1320

caatgcccac caccaccagt gtgccgcaca aggccgtggc ggtagggtat gtgtctgaaa 1380

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